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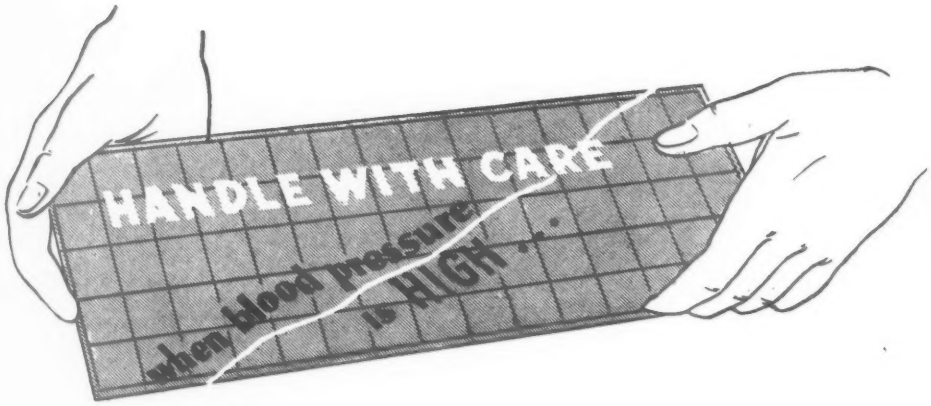

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THE AMERICAN JOURNAL
OF THE MEDICAL SCIENCES

JANUARY, 1944

ORIGINAL ARTICLES

THE NATURE OF GRAVES' DISEASE WITH SPECIAL REFERENCE TO ITS OPHTHALMIC COMPONENT*

By J. H. Means, M.D.

JACKSON PROFESSOR OF CLINICAL MEDICINE, HARVARD UNIVERSITY; CHIEF, MEDICAL SERVICES, MASSACHUSETTS GENERAL HOSPITAL, BOSTON, MASS.

(From the Thyroid Clinic of the Massachusetts General Hospital)

Around the turn of the 18th century there practiced in that charming little English city of Bath, made vivid for us by Booth Tarkington in his "Monsieur Beaucaire," a popular, fashionable but likewise astute physician, one Caleb Hillier Parry.

Like most of the great clinicians of the 18th and 19th centuries, Parry was not only a keen observer, but in addition an eloquent recorder of what he observed. On one occasion, for example, he wrote, "There is one malady which I have in five cases seen coincident with what appeared to be enlargement of the heart, and which, so far as I know, has not been noticed, in that connection, by medical writers. The malady to which I allude is enlargement of the thyroid gland." In further description of one of these, Parry states, "The eyes were protruded from their sockets, and the countenance exhibited an appearance of agitation and distress especially on any muscular exertion, which I have rarely seen equalled." Of the heart action he notes, "Each systole . . . . shook the whole trunk of the body."

Parry's[47] is the first adequate description of this disease, and it should by rights be called after him. Posterity has ordained, however, that the honor should be bestowed on others. In the English-speaking countries it falls to Robert Graves,[19] of Dublin, and in Germany, to von Basedow,[7] who was the last of the three to describe it. However, a more complete description of the eye phenomena was given by von Basedow than by either of the others.

You may well ask, Why make use of an eponym at all? My reply is that an etiologic label is still impossible because the etiology is unknown, and furthermore because I know of no descriptive term broad enough to fit all the cases which I believe come within the clinical entity. "Exophthalmic goiter" is not adequate because there

* A lecture delivered before the Alpha of Tennessee Chapter of Alpha Omega Alpha, Vanderbilt University, January 29, 1943.

is not always exophthalmos. There is not always goiter. "Toxic goiter" will not do for they are not always toxic. Graves' disease it still has to be. Within the group so termed, I shall include those cases of thyrotoxicosis with diffuse hyperplasia of the thyroid, with or without the type of ophthalmic involvement described by Parry, Graves and Basedow, and those cases with the characteristic ophthalmic involvement whether or not they be accompanied by thyrotoxicosis.

In other words, whereas the classic picture of Graves' disease includes both toxic goiter and certain peculiar ophthalmic manifestations, there are also certain cases belonging, I am sure, within the clinical entity, in which there is characteristic ophthalmic involvement with but little or no accompanying thyrotoxicosis. There may actually be hypothyroidism. My colleagues and I,[42,43] of late, have adopted the practice of subdividing our cases of Graves' disease into the classic type and the special ophthalmopathic type.* The latter includes what have variously been called in the literature "malignant exophthalmos,"[34] "exophthalmic ophthalmoplegia,"[8,12] "progressive postoperative exophthalmos with low basal metabolic rate,"[18] and so forth.[29,64,65,66,70] In the classic type, thyrotoxicosis and eye involvement are both present, and as the disease progresses the intensities of the two vary in parallel. Furthermore, in the classic type, the thyrotoxicosis is, from the therapeutic point of view, the major consideration. If it is relieved, the eye condition usually improves also without special treatment.

In the special ophthalmopathic type, on the other hand, the ophthalmopathy in its progression seems divorced or dissociated from the thyrotoxicosis; and from the therapeutic point of view the eyes, not the thyrotoxicosis, provide the chief indication for treatment.

I do not wish to imply that a sharp line can be drawn between the classic and special ophthalmopathic types of Graves' disease. Indeed, in a single case the picture may proceed from one type to the other, and in another case perhaps the subdivision cannot be made at all. However, there are cases, or stages in cases, where the subdivision can be made with ease, and I shall try to establish that it is of both physiologic interest and therapeutic importance to make it.

Etiology. Let us consider now for a few moments the general nature of Graves' disease. Is it infectious, neoplastic or a deficiency syndrome? Presumably none of these. What then? About all that can be said is that it is a breakdown of that balance of hormonic action which is necessary to the maintenance of health. We used to think of it in terms of disturbed thyroid function alone. Since the discovery of the thyroid stimulating hormone of the pituitary (TSH) it has been

* An ophthalmologic colleague has objected to my use of "ophthalmopathic" on the ground that such an adjective refers to the eyeball rather than to the orbit. I find, however, that ὀφθαλμός means eye, and that eye means the organ of vision. Since we have a separate word eyeball, eye must be more inclusive, and I regard it as proper to use the word as including the eyeball's adnexa as well as the globe itself. Also I am impressed by the fact that ophthalmologists in caring for patients include the adnexa within their field. Furthermore, it is to be noted that for years the term "eye signs" has been used with reference to all the ocular manifestations of Graves' disease. If eye, then why not ophthalmic? and if ophthalmic, why not ophthalmopathic?

evident that the pituitary, at least, among the other endocrines is also concerned in the morbid physiology of Graves' disease. But this is not all, for it has been shown that there are changes in the thymus, the musculature, and in the lymphatic and reticulo-endothelial systems as well. Altogether it is a complicated derangement.

What causes the breakdown? Why do some persons suffer from it and others not? It has been thought that shocking experiences, stresses and strains of living in one way or another pull the trigger and cause the explosion which is the disease. Certainly it is true that one can frequently get histories in which what may be called psychic traumata are rapidly followed by the development of the disease. So too, as Hertz and Means[22] have shown, metabolic insults such as rapid and great weight loss, intentional or otherwise, may act in similar fashion. However, in many other cases no such history can be elicited and the onset is gradual and insidious instead of explosive. Furthermore many persons, most in fact, sustaining such impacts do not develop Graves' disease. Instead they may develop peptic ulcer, hypertension, ulcerative colitis or a psychosis.

This makes one think that in addition to a precipitating factor it is necessary, for the production of Graves' disease, also to have a predisposed individual. How predisposed? By heredity? Perhaps. Certainly it is not uncommon to find families with a high incidence of Graves' disease running through several generations. But these are greatly outnumbered by those cases in which no family story can be obtained. My own guess is that Graves' disease represents a breakdown under stress or strain in a given individual's weakest part. Only a few of us are like Oliver Wendell Holmes' "One Horse Shay" in which every part was made just as strong as every other part so that no part could give out before another. The result, as you should recall, was that the whole affair suddenly completely disintegrated after 100 years of use. This is analogous to the nonagenarian who passes away peacefully without any evidence of disease.

Most of us are not built that way. According to the nature of our constitutions one of us may fail to adjust to his environment in his endocrine system and develop Graves' disease, another in his gastrointestinal tract and develop an ulcer, yet a third in his circulatory system and develop irritable heart or effort syndrome.

Very recently I saw identical twin girls of 10. Cynthia had developed definite, but mild, classic Graves' disease. Carol was apparently quite normal. Why, with identical constitutions and environments, had they not either both developed the disease, or neither. The only clue I could get was in the mother's statement that Cynthia had been infested with and treated for roundworms of some sort just prior to the development of her Graves' disease, while Carol had escaped. I am rather expecting, however, to hear that Carol has developed the disease also. In passing, it is interesting to note that Cynthia, in the few months she had been ill, had gained an inch and a half in height over her sister. This growth acceleration in thyrotoxic children was pointed out by Hertz and Galli-Mainini[21] in 1941.

The onset and the course of Graves' disease are most variable. Time will not permit even an enumeration of the various patterns. I shall merely remind you that it may start with either thyrotoxic manifestations or ophthalmic, or with the two simultaneously. The onset may be abrupt or gradual. There may be one or more spontaneous remissions and relapses. There is a definite tendency for the disease to be self-limited. It tends, as we say, to burn itself out. The total duration may be only months, or it may be, if untreated, many years. It may occur in either sex and at any age. Sometimes it goes into a low-grade, chronic form.

FIG. 1.—Graves' disease in one of twins. (Kindness of Dr. Nathan B. Talbot.)

Morbid Physiology. Of the morbid physiology of Graves' disease more is known than of its etiology.

The thyroid gland is certainly under abnormal stimulation. In many instances, it delivers to the blood stream an excess of its hormone. Hyperthyroidism results. This is manifested clinically by the familiar symptoms of thyrotoxicosis, and in the laboratory by increased basal metabolic rate and elevation of the protein-bound iodine in the blood. Histologically it is manifested by high columnar epithelium.

The nature of the stimulus to the thyroid is not clear. For years it was thought to be nervous. But now, I think, the weight of evidence is against any direct nervous stimulation of thyroid cells to secrete. It is clear, of course, that the anterior pituitary makes a thyroid stimulating principle (TSH) and that the administration of this material will cause hyperthyroidism in animals and man, indeed, a good imitation of Graves' disease, because exophthalmos may be produced as well. There is also good evidence that the thyroid hormone suppresses the action of the pituitary with respect to the production of TSH,[15,58,69] that lack of it stimulates it[56] and thus emerges a balance between the two glands to which Salter[59] has given the term "pituitary-thyroid axis." Furthermore Galli-Mainini[16] has made observations, *in vitro* with the Warburg apparatus, which indicate that the thyroid hormone inhibits the thyroid cells also. That is to

say, as the concentration of thyroid hormone rises in the blood, a depressing action will be exerted on the thyroid cell. This work is yet to be confirmed.

The rôle of the pituitary in clinical Graves' disease is far from established. There has been some tendency to regard the disease as primary hyperpituitarism with secondary hyperthyroidism. That this is an accurate conception, I think doubtful. There is no evidence that the anterior lobe is commonly hyperplastic in Graves' disease and only inconclusive evidence that there is an excess of TSH in the blood. However, even so, the gland may be putting out an increased quantity because the blood level of TSH would not disclose its rate of manufacture, but merely the relation between rate of entry and departure from the blood stream.

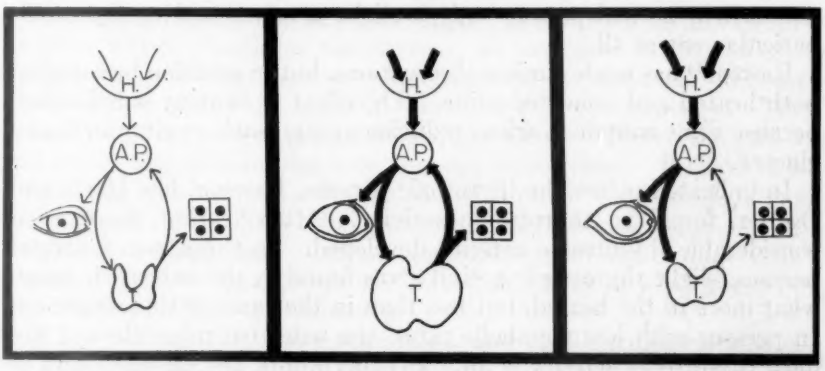

FIG. 2.—The pituitary-thyroid axis. In the *left-hand diagram*, as shown by arrows, a normally stimulated hypothalamus, *H.*, stimulates, in normal degree, the anterior pituitary, *A.P.*, to secrete its hormone which stimulates the thyroid gland, *T.*, to make its hormone which stimulates all tissue cells and causes an elevation in their metabolic rate. As further shown by arrows, the thyroid hormone also inhibits the *A.P.* and the thyrotropic hormone of the *A.P.* is believed to play some sort of direct rôle in the determination of the volume of orbital contents. In the *central diagram*, as shown by the heavier arrows, everything is intensified all around. The thyroid enlarges and hyperfunctionates, the ophthalmopathy develops. This is illustrative of what is believed to occur in classic Graves' disease. In the *right-hand diagram* we have the same, until the thyroid is reached. This gland, for reasons unknown, fails to respond to pituitary stimulation, at least to the same degree, hyperthyroidism is not produced, but the ophthalmopathy develops and progresses perhaps because sufficient inhibition of *A.P.* by *T.* is lacking. This is the state of affairs believed to exist in the ophthalmopathic type of Graves' disease.

If the mode of action of TSH on the thyroid cell be learned, light may be thrown on the nature of Graves' disease. Rawson[57] and his collaborators have made some important observations in this connection. In the first place, they found that if TSH be exposed as part of a substrate to thyroid cells growing in tissue culture, its physiologic activity is lost. This loss, however, they were able to show further, is the result of inactivation, not of degradation, of the hormone, because if treated with mild reducing agents such as glucose or vitamin C at the right temperature and pH, the physiologic activity could be restored. The hormone, under such circumstances is reactivated.

Physiologic activity is assayed by injecting the test material into day-old chicks and measuring the cell height of their thyroid epithelium. It looks, in other words, as though TSH affects the thyroid cell by acting as a catalyst on its oxidative enzyme system.

Rawson[54] also, by his tissue culture method, has observed the TSH-inactivating properties of various types of goiters removed surgically from patients. He finds that the thyroid tissue of thyrotoxic patients inactivates nearly all of the TSH added to the substrate, whereas equal amounts of normal thyroid tissue, obtained while operating on persons with parathyroid tumors, inactivates only one-half as much as that inactivated by the glands of thyrotoxic persons. In the case of non-toxic nodular goiters, there is no inactivation of TSH at all.

Some years ago, Hertz and Oastler[23] studied the thyrotropic activity of urine. In certain cases of myxedema they found a considerable amount, in normal persons only a slight amount, and in thyrotoxic patients none at all.

Rawson[53] has made similar observations, but in addition has studied both heated and unheated urine. The effect of heating is reduction, because urine contains various reducing agents, such as vitamin C and glucose.

In unheated urines the thyrotoxic persons, Rawson, like Hertz and Oastler, found no thyrotropic activity. After heating, however, a considerable thyrotropic activity developed. In the urine of normal persons, slight thyrotropic activity was found in the unheated, somewhat more in the heated, but less than in the cases of thyrotoxicosis. In persons with low metabolic rates, the unheated urine showed the most thyrotropic activity of all. In other words, the thyroid gland of persons with thyrotoxicosis has an increased power to inactivate TSH, and the greater total amount found in such cases suggests that there may be a greater production of TSH by the anterior pituitary.

In 2 cases of the special ophthalmopathic type of Graves' disease, Rawson found high TSH activity in the unheated urine. He concluded that in these, as in the thyrotoxic type, the anterior pituitary overacts with regard to TSH secretion, but for some reason unknown, the thyroids in these cases cannot respond to it, and TSH in consequence gets by, not inactivated, to be excreted in the urine.

Another approach to thyroid physiology has been provided by Hertz and his co-workers.[24–28] This depends upon the use of artificially radioactive iodine, which can be used in a tracer method of studying the function of the thyroid gland. The thyroid hormone contains iodine, and it is an ingredient essential to physiologic activity; therefore, thyroid cells in order to produce the hormone must have a supply of iodine. Lacking this, be the tissue ever so hyperplastic, no physiologically active thyroid hormone can be produced. Cyanates, for example, have been found to interfere, in some way not yet completely understood, with the elaboration of the thyroid hormone, and we have obtained a biopsy in the case of a man who developed a goiter while receiving potassium sulfocyanate as treatment for hypertension. The thyroid was wildly hyperplastic, but the patient was hypothyroid![55]

By means of the radioactive (labeled) iodine, it has been found that the hyperplastic thyroid collects administered iodine at a much faster rate than does the normal, and this holds whether the increased effort of the thyroid cells to make hormone is the result of stimulation by TSH or through some other agency, such as a stoppage or bottleneck in the assembly line of hormone production, which is what we conceive happens in the case of cyanates and some similar substances.

Of thyroid physiology in Graves' disease at least it can be said that the cells of the thyroid are hyperfunctioning, or trying to hyperfunction, and that a factor in this functional alteration is an increased ability to inactivate TSH. It is to be presumed that the anterior pituitary, even though not visibly hyperplastic, makes an increased amount of TSH, because an increased amount, as compared with the normal, is found in the inactivated form in the urine.

Thus in Graves' disease we have evidence of an increased flow of the hormone which stimulates the thyroid, an increased avidity of the thyroid for iodine, a necessary ingredient of its hormone, and an increased flow of thyroid hormone which inhibits the pituitary. The pituitary-thyroid axis is geared at a higher level. Where the factor that causes this shift impinges on the axis, is not clear. Perhaps it is in the form of a nervous influence routed through the hypothalamus to the anterior lobe. Also other endocrines may play some minor rôle.

Ophthalmic Component. Let us now focus on the ophthalmic component of Graves' disease, indulge, in fact, in a little of what might be called endocrinologic ophthalmology.

In the 19th century there were many and excellent descriptions of the disease in general and of the eye signs in particular. Parry had noted the wild, staring appearance and the protrusion of the eyeballs. Von Basedow had linked proptosis or exophthalmos, violent heart action and goiter as the essential framework of the syndrome. After his home town, it came to be known in Europe as the Merseberg triad. The names of various ophthalmologists and others became attached to various special manifestations that accompanied the protrusion: Dalrymple's sign being retraction of the upper lid; von Graefe's, lag of the lid behind the globe in downward rotation; Stellwag's, widening of the palpebral slits and infrequent wrinkling; Moebius' sign, difficulty in converging the eyeballs in attempting to fix near objects; Jellinek's sign, pigmentation of the eyelids; Kocher's sign, in upward rotation, movement of the lid ahead of the eyeball (this sign is practically von Graefe's in reverse, perhaps we may be permitted to call it globe lag); and Joffroy's sign, absence of brow wrinkling on upward gaze with head lowered.

To this galaxy of 19th century eponymously labeled manifestations we may add yet others, which more recently have seemed important, such as edema of the eyelids, chemosis or edema of the global conjunctiva, ulceration of the cornea, epiphora, and visual defects such as diplopia, diminution in acuity of vision and (found by us in 1 case only) bitemporal hemianopsia.

I should like to make it clear that, in case-taking, there is no virtue

in merely recognizing and recording individually certain sonorously named eye signs. Indeed, I think there is but little good reason for burdening the memory with which man's name goes with which phenomenon. The real objective should be to size up the ophthalmic situation *in toto* and interpret its significance. What can one conclude, on the basis of observed phenomena, about what is actually going on in the patient? Is the lesion advancing, regressing or remaining stationary? Those are the things that must be known if treatment is to be planned intelligently.

Special attention to isolated eye signs is only indicated if, and when, they have some special significance. The last category of signs that I mentioned, edema of lids and conjunctiva, does, as will appear later, have a very special significance There is a possibility that lid retraction, lid lag and globe lag (these are really but different aspects of a single abnormality, namely spasm of the levator palpebræ superioris

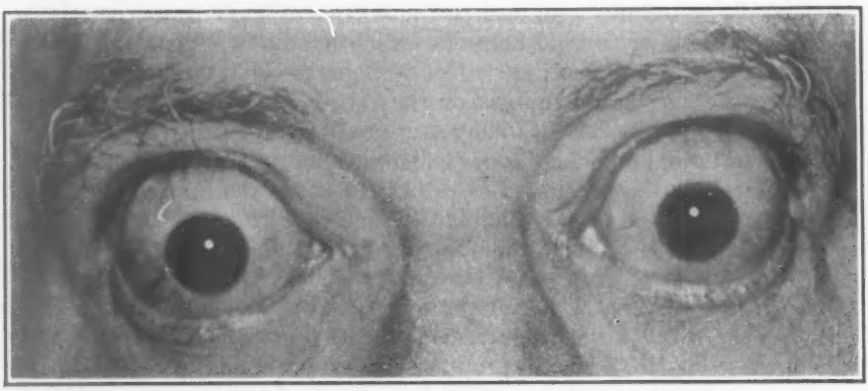

FIG. 3.—Graves' disease of special ophthalmopathic type in a man of 55 years. BMR −5 on iodine. No thyroidectomy. Extreme lid retraction, moderate proptosis O.U. Slight lid edema, no chemosis. (U. 301702.)

muscle) also have a special significance. All the other eye signs may be said to be related to, and determined by, the state of affairs within the orbit. The ophthalmic component of Graves' disease is an affliction of extraglobal orbital contents, not primarily of the eyeball. The eyeball is pushed forward and its membranes engorged because of what is going on behind it in the orbit, or the muscles controlling it, and the eyelids, or both, are the seat of pathologic alteration.

The first point to settle in the person suspected of having Graves' disease is whether eye signs, any eye signs, are present or absent. In early Graves' disease the only detectable abnormality may be staring expression of the eyes and lid lag. There may be no protrusion of the globes at all. We may, indeed, measure their position with an exophthalmometer and find it quite within the limits of normal. Despite the lack of exophthalmos, however, the staring, wild look is visible across the room and is close to pathognomonic as far as diagnostic import goes. It is due to the widening of the palpebral fissure,

Dalrymple's sign, with exposure of the white of the eye above the iris. A similar appearance might be found in states of real terror, or it can be produced voluntarily, that is to say, faked. When it is not faked nor the result of terror, then the sign is very strong evidence for Graves' disease. Often it is asymmetric or unilateral, as has been emphasized lately by Pochin.[50,52]

Years ago von Graefe pointed out that lid lag is independent of exophthalmos.* He believed it to be of sympathetic origin due to an affection of the sympathetic innervation of the levator palpebræ superioris. Pochin,[51] however, maintains that while the lower lid is lowered by sympathetic stimulation, it is raised relative to the cornea when the upper lid is retracted by the levator palpebræ superioris. This is a striated muscle and is innervated, not by the sympathetic, but by the oculomotor nerve. The sympathetic overactivity theory of lid retraction, therefore, according to Pochin, is untenable. Indeed, I have concluded after searching the literature that the sympathetic has little if anything to do with any of the ophthalmic manifestations of Graves' disease. In certain types of experimental exophthalmos in animals, yes; in human Graves' disease, no. Nor do I believe that sympathectomy offers anything in the treatment of human exophthalmos, Jonnesco[31] to the contrary, notwithstanding.

At the onset of Graves' disease, thyrotoxic manifestations may be the first to appear, or the ophthalmic, or the two may appear together. Relationships of this character were noted as early as 1860 by Holthouse (in discussion of C. H. Jones[30]). As I have already indicated, thyrotoxic and ophthalmic components may run parallel courses, or vary inversely or independently. These variations serve as a basis for the separation of cases into types.[42,43] Furthermore, either thyrotoxic or ophthalmic manifestations may be lacking, but in the absence of both one could not make a diagnosis of Graves' disease.

I have been particularly interested of late in those patients who complain of their eyes first. Often they go first to the ophthalmologist and sometimes he fails to appreciate that they actually have Graves' disease, not a local ophthalmic disorder. Early this month, for example, I saw a doctor's wife who last May thought she had eye strain and consulted an ophthalmologist. He told her she had muscle trouble with poor convergence. His observation was correct, but

* After this lecture had been delivered at Nashville, Dr. Barney Brooks told me that I had ignored the action of the lids. He believed that one cannot have lid retraction without some degree of protrusion, that the inevitable effect of a widened aperture is some forward movement of the globe due to diminution in the restraining action of the lids. Soon afterward, Dr. David G. Cogan, at the Howe Laboratory of Ophthalmic Research, was good enough to make some observations on normal people (including myself). By means of ophthalmometric measurements he found that when the lids are held open wide with a speculum there is no protrusion beyond the limit of accuracy of the instrument, which is 2 mm. Some of the subjects, however, during voluntary retraction showed protrusion of 3 mm. Cogan concluded that it does not appear from this small series that the lids exert an appreciable effect in retaining the normal eyeball in the orbit. The active contraction of the levator, however, may force the globe out slightly through pressure on orbital contents. In view of these findings I cannot believe that widening of the aperture is an important factor in the causation of the proptosis of Graves' disease.

apparently he failed to recognize that this could be Moebius' sign in Graves' disease. Soon after she developed goiter and thyrotoxic symptoms. Most of the patients who go first to the ophthalmologist or eye clinic come to fall within our special ophthalmopathic group, but not all.

As Graves' disease progresses the ophthalmic picture may develop in various ways. In addition to lid retraction, proptosis may appear and increase in intensity. It may be asymmetric, as in the case of lid retraction, but it is rarely completely unilateral. I have often seen patients who at first glance appeared to have but one eye sticking out. However, on careful examination, some abnormality, perhaps only lid retraction or a lid lag, could be detected on the other side. If it can be demonstrated that eye signs are bilateral, even though asymmetric, it serves to rule out such lesions as postorbital tumor or arteriovenous aneurysm of the cavernous sinus, which would give a purely one-sided

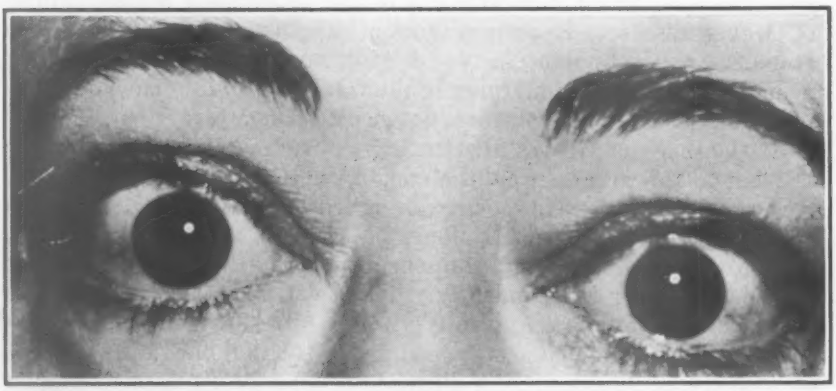

FIG. 4.—Graves' disease with development of progressive ophthalmopathy after thyroidectomy in a woman of 42 years. BMR −34 when picture taken. Lid retraction and proptosis very moderate, but edema and conjunctival injection marked. Chemosis present. Marked irritative symptoms, tearing, burning and so forth. (U. 252602.)

proptosis. Of other conditions than Graves' disease which can cause bilateral exophthalmos, we have encountered only skull deformities with shallow orbits, such as oxycephaly, Hand-Schüller-Christian's disease, rarely myasthenia gravis and possibly certain hypertensions. In brief, the finding of bilateral eye signs of the Graves' variety is overwhelming evidence in favor of that diagnosis.

If the ophthalmic component of Graves' disease is of systemic origin, why, it may be asked, is it often so markedly asymmetric? To that I can give no adequate reply beyond saying that the evidence in favor of systemic origin is very convincing. As the eye lesion develops, asymmetry may either diminish or increase. I have seen cases in which first one eye was the more prominent, then later the other caught up with and even passed it.

In evaluating the ophthalmic status in any patients with Graves' disease, the congestive and dropsical phenomena are very important.

Compare, for example, the case of a patient, who had classic Graves' disease years ago and has permanent residual exophthalmos, with that of one who has just started on the course of what we call the special ophthalmopathic type. In the former, the pure proptosis may be very marked, but there will be little, if any, lid retraction, and no edema of lids or conjunctivæ, or congestion, and in all probability no ocular symptoms. The eye condition here is quite stationary and inactive. It is, in fact, an end-result. In the latter case, the picture is quite different. The eyes have a sore and swollen look. The actual proptosis may be less than in the former, but the lids are edematous, the conjunctivæ are injected and chemotic, and all these tend to make his general appearance much more impressive, or even alarming, than in the case first described. The patient with the latter picture will have plenty of ophthalmic symptoms: tears, smarting, photophobia and so forth. One patient said that his eyes felt like golf balls. In another

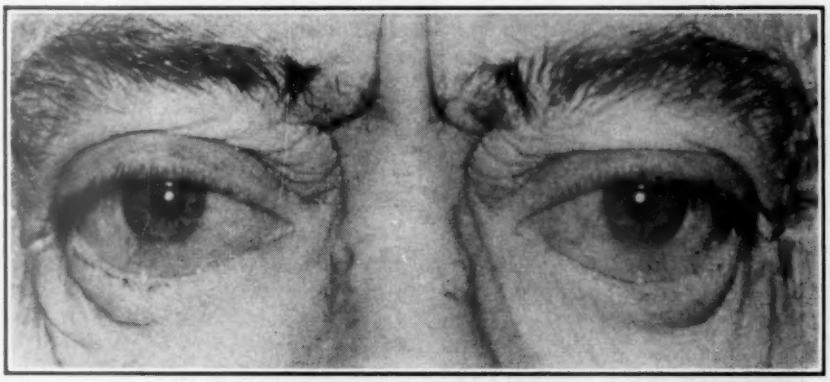

FIG. 5.—Graves' disease with development of a malignant type of ophthalmopathy 3 years after thyroidectomy for thyrotoxicosis, in a man of 54. BMR +5 off iodine—later to −15 on iodine. No lid retraction and therefore proptosis, which was of considerable degree, was obscured. Marked injection and chemosis of conjunctivæ. Later developed corneal ulcerations and required orbital decompression O.D. (U. 292087.)

patient the chief complaint may be the result of muscular weakness: diplopia or inability to fix near objects, difficulty in reading, and so forth. This type of ophthalmopathy represents an active process.

When Graves' disease is treated by partial thyroidectomy, the eyes often lose much of their staring appearance rather rapidly. The exophthalmos appears to subside. However, Soley[63] has shown that usually after operation actual measurement shows that, for a time, exophthalmos actually increases. The improved appearance is to be attributed to decline in lid retraction. In the classic type, however, ultimately the exophthalmos may decrease, although, as Galli-Mainini[17] has shown, it seldom completely disappears. Sometimes the eyes come to rest permanently in a markedly forward position, as in the case described earlier.

In those cases which fall in the special ophthalmopathic group, the eye condition becomes aggravated after operation and the progress of

the lesion may be such as to justify the appellation of malignant exophthalmos. Edema of the lids and chemosis of the conjunctiva become extreme. Ulceration of the cornea sets in, vision, and indeed the eyeball itself, is endangered. A truly terrible picture of bulging, inflamed eyes protruding through swollen lids is presented. The patient cannot close his lids, and this aggravates the inflammation through the irritating effect of drying of the cornea.

We have come to believe that when a hint that the ophthalmopathy may follow such a course is obtained before thyroidectomy is done, the most important indication for treatment is to refrain from removing the thyroid. It is significant, we believe, that in cases of malignant exophthalmos in which it finally becomes necessary surgically to decompress the orbit in order to save the eye, there has previously been a thyroidectomy in a very high percentage of cases. In other words, it is (with very few exceptions) only the post-thyroidectomy cases in

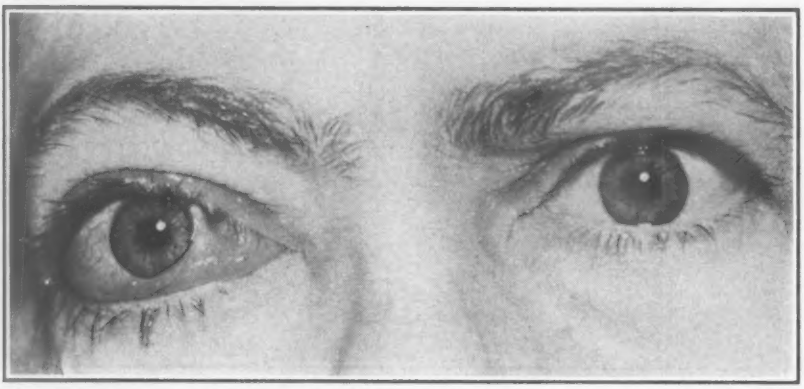

FIG. 6.—Asymmetric ophthalmopathy developing in a woman of 51 who had had thyroidectomy for Graves' disease 5 years earlier. She was also diabetic. At time picture was taken she was receiving iodine, thyroid and insulin. Her BMR was 0. No lid retraction, but moderate proptosis and extreme chemosis and conjunctival injection O.D. Her right orbit was treated by irradiation and she made a marked improvement. (U. 315242.)

which such serious eye conditions develop. Occasionally patients, who have had the disease in classic form and recovered with or without thyroidectomy, turn up some years later with a recrudescence which assumes the special ophthalmopathic type rather than the classic.

Pathogenesis of the Ophthalmic Component. Let us now consider the pathogenesis of the ophthalmic component, and its relation to that of the disease *in toto*. There are, of course, two main approaches: the study of the naturally occurring disease in man and the endeavor to reproduce it experimentally in other species. Also the problem can be divided into that of the local intra-orbital mechanism and that of what lies behind it.

On the former question, observations on humans are probably more valuable than experiments on animals. The difficulty with animals is that the mechanism of exophthalmos may be quite different from what

it is in humans. For example, as long ago as 1858, Müller[45] distinguished two groups of smooth muscles in the eyes of mammals, periorbital and palpebral. In some species the periorbital is so well developed as to constitute a slinglike muscle capable of pushing the eyeball outward on sympathetic stimulation or on the exhibition of sympathomimetic drugs (Brunton[9]). This has been called Müller's muscle. It is rudimentary or absent in man and plays no rôle in human exophthalmos.

In 1904, MacCallum and Cornell[36] produced exophthalmos in dogs by obstructing the outflow of blood through the orbital veins. This was followed by the development of edema of the orbital tissues which further enhanced the exophthalmos. Again it is unlikely, as these authors admit, that such a mechanism plays a rôle in human Graves' disease.

A better clue to the situation in man is provided by the observations of the English ophthalmologist, Moore,[44] in 1920. In a case of malignant exophthalmos he incised the conjunctiva and removed as much orbital fat as possible to relieve tension. He remarked that the fat "seemed edematous, and in particular the inferior, internal, and external recti muscles were exposed, for a considerable distance, and these, instead of being thin, flat, ribbon-like muscles, such as one becomes familiar with in squint operations, were greatly swollen fusiform bellies apparently from œdematous infiltration, not quite so stout as the last joint of one's little finger." So far as I know, Moore's is the first study of the pathology of the living in this condition. He also obtained an autopsy in 1 case of exophthalmic goiter and concluded that, since the exophthalmos remained marked after death, it could be due neither to sympathetic irritation nor to venous engorgement, but rather to edema of orbital contents, and perhaps increase in orbital fat. Thomson,[67] in 1924, also upheld the edema theory, saying "exophthalmos in exophthalmic goitre is due to a localized edema in the posterior part of the orbit; which, for some unknown reason, if sufficiently prolonged, is followed by connective tissue proliferation and permanent thickening of the orbital tissues." We might suggest that the edema, like that in the legs of patients with long-standing cardiac failure, becomes brawny. The German, Unverricht,[68] in 1925, also supported this theory.

A really important contribution to the pathology of the condition is that of Dudgeon and Urquhart,[11] in 1926. These investigators examined extrinsic eye muscle, heart muscle and skeletal muscle in 9 postmortems on exophthalmic goiter patients. Lymphorrhages, great infiltrations with lymphocytes and some plasma and endothelial cells, were found in the muscles of 8 of the 9 cases. They were most marked in the ocular muscles.

The first extensive observations of the pathology of the living in the exophthalmos of Graves' disease are those of Naffziger,[46] who in 1931 devised the operation of orbital decompression. On opening the orbit through the cranial cavity, Naffziger found the orbital contents bulged, due entirely, he thought, to a great increase in the size of the

extrinsic muscles (3 or 4 times their normal volume). Microscopically, the muscles showed edema, round cell infiltration often with a perivascular pattern, loss of architecture, increase in fibrous tissue with areas of hyalinization and fragmentation, and destruction. Friedenwald[13] confirmed Naffziger's findings in 1932.

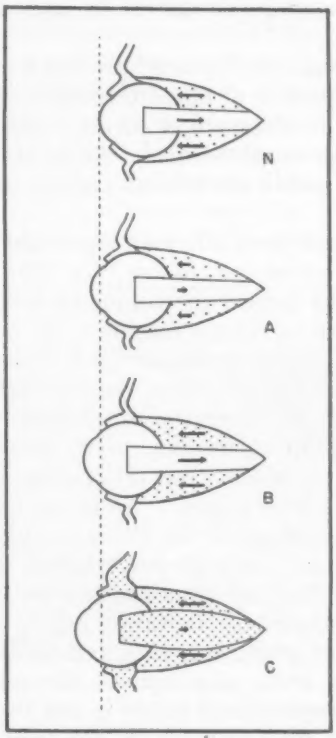

FIG. 7.—Galli-Mainini's schemata representing the mechanism which may be involved in exophthalmogenesis. "The lids, the eyeball, one muscle and the intra-orbital tissue are represented. The arrows indicate the direction of pressures and tensions. The dots represent the amount of fluid in the tissues. In Schema N is shown the situation in health. The pull of the muscle is of the same magnitude as the tissue pressure and balances it, so that the position of the globe is that found in normal persons, indicated in these schemata by the interrupted line. Schema A shows the first step in the development of exophthalmos. The muscle has weakened, exerts less tension, indicated by the shortened arrow. The tissue pressure therefore forces the eyeball forward. As this happens tissue pressure falls and fluid passes from the capillaries to tissue to occupy the space, until a new equilibrium is reached with the muscles stretched and the eyeball protruding—Schema B. In Schema C is shown the situation in progressive exophthalmos in which a weakened and degenerate muscle cannot withstand the tissue pressure and in its turn becomes edematous, as do the lids and surrounding tissues."

Thus it seems established that a part of the immediate cause of exophthalmos in Graves' disease is swelling of the orbital contents. In the malignant cases, swelling of the muscles may be the chief factor. In others, edema of the other orbital contents may play a rôle.

That the whole phenomenon of proptosis may not be due to increased push upon the globe, however, is suggested by Galli-Mainini.[17] He points out that there is also the factor of decreased pull. "The eye-

ball," he says, "fits the orbit somewhat as a cork does a bottle, and it is maintained in position by the action of two pressures working in opposite directions. One of these is the pressure exerted by the tension of muscles, which tends to pull the eyeball backward; the other is the tissue pressure of the orbital contents, which tends to push the eyeball forward. The two are in such balance in health as to maintain a constant volume of orbital content, and at the same time to secure normal movement of the globes and circulation of the orbit."

The cork and bottle simile is good, and one should perhaps further specify a bottle of champagne. Here we have a cork held in by wires against a high pressure within the bottle. Cut the wires and out comes the cork. Weaken the muscles and out comes the eyeball.

That the ocular muscles may be weakened in Graves' disease, from the kind of pathologic change found by Moore, Dudgeon and Urquhart, Naffziger and Friedenwald, seems a fair inference. Also there is abundant evidence that generalized weakness of striated muscle is characteristic of the malady.[2,5,33,37] That a tendency to water retention also exists in Graves' disease seems likely in view of the frequent occurrence of moderate edema without evidence of heart or renal failure. Why in a general condition should edema formation be chiefly manifested locally in the orbit? This may be because it is only in the orbit that we have the cork and bottle set-up. The combination of increased tissue pressure and decrease in the force opposing it (the pull of the extrinsic muscles) is only to be found there. Galli-Mainini visualizes the sequence of events as follows: A weakening of the muscles upsets the equilibrium between the muscle pull and tissue push of health. The globe moves forward. As this happens, tissue pressure falls and fluid passes from capillaries to tissue to occupy the space, until a new equilibrium is reached with the muscles stretched and the globe protruding. If this situation is stabilized we have the sort of permanent inactive residual exophthalmos which I have described. On the other hand, it may become progressive and as the muscles weaken they cannot withstand the tissue pressure and in turn become edematous, as do the lids and surrounding tissues. According to this concept, edema of the lids, and such phenomena as accompany it (chemosis and injection of the conjunctiva), would always denote an active or progressive process, and absence of them, as Haines[20] has pointed out, would indicate an inactive or receding condition.

On the purely experimental side we have a number of leads. Many investigators report the production of exophthalmos in animals with extracts of the anterior pituitary. The first of these, I believe, was Schockaert,[60] who produced thyroid hyperplasia and exophthalmos in young ducks, in 1931. I remember his telling us about it on a visit to our laboratory and causing much excitement thereby. A year later, Loeb and Friedman[35] obtained exophthalmos in guinea pigs by injecting an acid extract of anterior pituitary of cattle. As this exophthalmos receded if injections were discontinued and under narcosis or postmortem, it differed from the exophthalmos of Graves' disease in humans. Marine and Rosen[41] next showed that exophthalmos can be produced by TSH injection even more easily in thyroidec-

tomized guinea pigs than in intact ones. In 1934, Friedgood[14] observed that TSH-induced exophthalmos in guinea pigs became worse during a refractory period, and was especially well marked in animals in which a low basal metabolic rate developed. This finding fits very well with what we observe clinically.

Smelser,[61] in 1936, confirmed Marine and Rosen's finding that thyroidectomy enhances the exophthalmos induced in guinea pigs by TSH injections. He also showed that if one side be sympathectomized in advance the exophthalmos is less on that side. This might not hold in the human. Smelser,[62] furthermore, found that the orbital fat and connective tissue of his exophthalmic pigs was edematous and the muscles were increased in size. Paulson,[48] of the Mayo Clinic, in 1937, reported similar findings in the orbit and in the extra-ocular muscles, changes similar to that described by Naffziger in humans. Paulson found no changes in other skeletal muscles except the orbicularis oculi in which, in a few instances, degeneration of the fibers was noted. In 1939, Paulson[49] published further work showing the production of marked retroglobal edema in TSH-injected thyroidectomized guinea pigs, which he believed responsible for the ocular protrusion. He also, this time, obtained definite degeneration of skeletal and cardiac muscle. Iodine had no effect on the development of exophthalmos, although it did lessen the thyroid hyperplasia induced by TSH. He concluded that iodine acts on the thyroid gland and is not directly inhibitory or antagonistic to TSH. We entirely agree with this interpretation. In 1940, Aird[1] showed that after several months of injection of TSH the exophthalmos of guinea pigs persists despite cessation of injection and after narcosis or death, attributing this to the enlargement of the muscles. In discussion of Aird's paper, Marine pointed out that there were two types of exophthalmos: that without and that with organic change in the orbit. The second has not been produced in the rabbit, only in man and the guinea pig, and for the most part after thyroidectomy.

Marine[40] believes that in addition to the anterior pituitary, the gonad plays an important rôle in the production of exophthalmos. For example, castration of male rabbits caused an existing exophthalmos to regress. The exhibition of testosterone propionate to these same rabbits caused the exophthalmos to recur. Since these observations were made on rabbits, they may be quite inapplicable to man. However, it is of interest to note, in this connection, that those cases of Graves' disease which we have called the special ophthalmopathic type, are relatively more numerous in males than are those of the classic type. Marine also believes the adrenal cortex may be involved in the endocrine hookup of Graves' disease, though the connection seems obscure.

In the light of all these various studies on the relation of TSH to exophthalmos, it is interesting to recall that Cannon[10] and his coworkers, back in 1915, produced in 4 cats a syndrome very like human Graves' disease by joining the phrenic nerve with the cervical sympathetic. These cats developed exophthalmos and hyperthyroidism. They also had hyperplastic adrenals. The authors concluded that the thyroids of their animals had become overactive from direct ner-

vous stimulation, but the weight of modern evidence favors the interpretation that actually it was the anterior pituitary which was nervously stimulated and the thyroid, in turn, humorally by the pituitary. Such a theory accounts for the adrenal hyperplasia also.

Of late there has been much interest in the effects of cyanates upon the thyroid in man. It has been found by several observers,[6,32,55] including my colleagues and me, that patients, treated for a long time with potassium sulfocyanate for hypertension, may develop a syndrome characterized by hyperplastic goiter with hypofunction. In 1 of our cases there was also slight, but definite, exophthalmos. Furthermore, thyroid hyperplasia has been produced in animals by other sulfur-containing substances such as thiourea, thiocarbamide and the sulfonamides.[3,4,38,39] Rawson has very recently been treating rats, in our laboratory, with sulfathiazole in order to produce hyperplastic goiter. He gained this objective satisfactorily, but also found that his rats developed very definite exophthalmos which persisted after death. It is not clear precisely where such agents impinge on the pituitary-thyroid axis, but the weight of evidence is that it is on the thyroid directly, imposing some obstruction to the completion of the elaboration of thyroid hormone. The organism thus becomes hypothyroid, which serves as a stimulus to the pituitary to increase its output of TSH, which, in turn, causes the thyroid to become hyperplastic and the eyes to become protruded. The hyperplasia of the thyroid, however, is ineffective because the obstruction to completion of thyroid hormone persists.

Summary. What can we conclude about the nature of Graves' disease from the various bits of evidence which I have cited? Not much, perhaps, but yet something.

Seemingly the malady is determined, to some degree, by inherited constitution, plus, very likely, some environmentally imposed stress which the given constitution cannot take without departing from the healthy state.

Hyperplasia of the thyroid seems to be a characteristic feature. Usually, but not always, there is production of hyperthyroidism.

The behavior of the thyroid is very likely secondary to humoral stimulation from the anterior pituitary. The overactivity of the pituitary may well be due to a nervous impact reaching it *via* the hypothalamus.

The response to whatever the stimulus be, which sets off the disease, may follow a different course in one individual from that in another.

The ophthalmic portion of the disease is both interesting and mysterious. It is of great importance from the point of view of therapeutics.

It is fairly evident that swelling of the extraglobal orbital contents, especially the extrinsic muscles, is a major immediate cause of exophthalmos. Weakness of the muscles, with decreased back pull, is probably another—indeed, the latter may be the first to become operative.

The cause of these local changes, and what determines their progression, or recession, is not so clear. Certainly they are not the result of thyrotoxicosis *per se*. The thyroid hormone is diuretic in action. It should decrease swelling, not cause it. Considerable evidence supports

the view that an excess of thyrotropic hormone has something to do with the ophthalmic pathogenesis, but the relationship is probably not a simple one.

Why some cases follow what I have defined as a classic course, others an ophthalmopathic one, may be related to differences in the response of the thyroid cells to the action of TSH. In the classic type, TSH stimulates the cells, and they produce an excess of their hormone. In this process, TSH is inactivated and, as Rawson has shown, is excreted in that form in the urine. In the ophthalmopathic type, TSH is not inactivated to the same degree, the production of thyroid hormone is not increased and TSH is excreted in its active, as well as inactive form in the urine. At the same time, excess of TSH aggravates the ophthalmic situation.

I have said but little of treatment, but in closing I urge you, in planning treatment in this disease, to keep the morbid mechanism, in all its known aspects, in mind. Particularly, realize that the best therapeutic program may be quite different in one case from what it is in another. While subtotal thyroidectomy may be the best treatment we have at present for many patients, in other cases it is not only not helpful, but very likely harmful. To accept routine subtotal thyroidectomy as good enough treatment and not seek for better, is not a truly scientific attitude. This is the thought I should like chiefly to leave with you.

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TREATMENT OF ADDISON'S DISEASE WITH PELLETS OF DESOXYCORTICOSTERONE ACETATE*

BY REGINALD A. SHIPLEY, M.D.
CLEVELAND, OHIO

(From the Department of Medicine of Western Reserve University, School of Medicine, and the Lakeside Hospital)

DESOXYCORTICOSTERONE is the only adrenal cortical steroid which has been prepared synthetically, and hence is the only pure cortical principle available in a quantity sufficient for therapeutic use. Compared to the other adrenal steroids desoxycorticosterone possesses a high

* The desoxycorticosterone acetate (cortate), both in the form of pellets and dissolved in oil, was generously provided by the Schering Corporation through the courtesy of Dr. W. H. Stoner.

potency in maintaining the life of adrenalectomized animals, and exceeds all others in its capacity to stimulate retention of sodium chloride.[12] It has the practical advantage of being less expensive than cortical extract. One of its disadvantages, however, is the danger of overdosage. Edema, hypertension, cardiac failure, and paralytic states have been observed when excessive quantities of the compound were administered.[5,7,18,23,28] Furthermore, desoxycorticosterone lacks any appreciable capacity to influence carbohydrate metabolism,[16,17,27] and it fails to restore certain physiologic defects which follow adrenalectomy in animals.[9,24]

Despite its shortcomings this crystalline compound offers the unique advantage that it may be compressed into pellets, which, when implanted subcutaneously, give up the hormone to the tissues slowly and evenly so that therapeutic effects may be sustained for many months. The convenience to the patient, the absence of fluctuations in hormone absorption associated with injections, and the avoidance of all the uncertainties of self-medication are obvious points in favor of a preparation administered in pellet form. Moreover, Thorn, Greif, Coutinho, and Eisenberg[29] have pointed out that desoxycorticosterone when given in this manner is more effectively utilized than when given by any other method. Recently Thorn, Dorrance, and Day[30] have reported a series of 148 patients in which the results of treatment with pellets were, in general, very favorable. Sixty-one of these patients were under the direct observation of the authors. A number of other favorable reports have also appeared,[2,3,8,10,11,13,14,20,22,23,25] but these publications, for the most part, present data on only 1 or 2 patients who were observed for a relatively short period of time.

Pellet preparations of desoxycorticosterone acetate are not as yet available for general clinical use. The implication, therefore, exists that evidence pertaining to the desirability of pellet therapy is incomplete. Objections to such therapy might include not only an uncertainty as to its efficacy but also a fear of the effects of overdosage which cannot be alleviated without surgical removal of the pellets. In addition to questions of efficacy and hazard, clarification is also needed in relation to problems bearing on the type of pellet to be used, the number of pellets which should be implanted, their rate of absorption, and their maximum effective life.

The present report includes observations made on 7 patients with Addison's disease who have received treatment with pellets of desoxycorticosterone acetate. The diagnosis was established in each case by the presence of a typical clinical picture of the disease along with chemical studies which usually included a chloride excretion test[1] or water test[21] (Table 1). In 3 patients who have died there was confirmation of the diagnosis at autopsy.

Method of Insertion of Pellets and Number Implanted. Two types of pellets have been used. The first was flat and disk-like, measured approximately 3 x 9 mm., weighed 135 to 150 mg., and had a surface area of approximately 200 sq.mm. The other type of pellet, which is the one being used exclusively at present, is in the shape of an elongated

cylinder, measures 3.2 x 8.2 mm. and weighs 75 mg. Its surface area is 100 sq.mm. and volume 65 c.mm.

TABLE 1.—DIAGNOSTIC DATA

	Age	Sex	Concentration of Cl chloride excretion test (mg. per 100 cc.)	Water test: Night volume (cc.)	Water test: Maximum morning volume (cc.)	Water test: "A"	17 ketosteroids mg. per day	Electrocardiogram
Case 1 (A. G.)	35	M	...	500	40	2	5	Normal
Case 2 (F. G.)	62	F	...	..	..	..	2	Normal
Case 3 (G. H.)	47	F	205	..	..	..	..	...
Case 4 (G. L.)	35	F	346	197	50	3	5	Prolonged Q–T interval
Case 5 (J. B.)	28	M	...	435	85	5	5	Prolonged Q–T interval
Case 6 (M. W.)	30	F	...	300	50	5	3	Prolonged Q–T interval
Case 7 (R. P.)	33	M	274	1050	375	6	9	Prolonged Q–T interval

The pellets were inserted in the infrascapular region through an incision about 1 cm. long. Pockets radiating in various directions were made with a hemostat beneath the skin in the subcutaneous tissue. The pellets were then gently placed in position with smooth pick-up forceps. As many as 6 pellets could easily be inserted through one incision. There were no infections and in no cases were pellets spontaneously extruded. When the old pellets had been in place long enough to have undergone considerable absorption, they were removed, weighed, and new pellets inserted. There was usually very little tissue reaction around the pellets at the time of removal. A thin fibrous capsule, approximately 0.2 mm. thick was present and there was a variable degree of cellular infiltration just outside of the capsule.

Thorn has recommended that before pellets are implanted for the first time and at successive reimplantations the requirement of the patient should be determined by a preliminary trial with daily injections of the compound in oil.[30] He has calculated that one 125 mg. pellet should be inserted for every 0.4 to 0.5 mg. of hormone required by daily injection. Inasmuch as the pellets used in the present series of cases were not identical with those employed by Thorn, this calculation would not be applicable. A precise estimate of the optimum dose of this compound for a given patient is difficult to determine because a definite clinical or chemical end-point of response is lacking. Very small doses of hormone may be adequate to maintain normal electrolyte and water metabolism and yet not impart a satisfactory feeling of well being or restore muscular strength and endurance for work. In some patients an increase in the dose will bring about a substantial increase in subjective improvement, but in others signs of overdosage intervene and this end is not attained. On the other hand, many patients are well controlled and do equally well on doses of hormone varying as much as two-fold in amount.

In view of the difficulty encountered in determining precise dosage and in order to eliminate a rather impractical period of prolonged preliminary observation the patients in this series were implanted with pellets immediately after the period of diagnostic study. If within a few weeks or months there was not a degree of improvement which

seemed satisfactory, additional pellets were inserted. The optimal dose was considered to be that amount of hormone which, without signs of overdosage, maintained normal levels of plasma chloride, blood urea nitrogen, hematocrit, and blood pressure, and which gave a maximum degree of subjective improvement with moderate gain in weight. The initial dose was one or two 150 mg. pellets or two to four of the 75 mg. size. The maximum number eventually implanted in any patient to date has been four of the type weighing 150 mg. and six of the kind weighing 75 mg.

After leaving the hospital the patients were allowed free choice of food. The amount of salt ingested was derived only from that which was intrinsic in the foodstuff plus that which they chose to add to suit their tastes. This total varied from 8 to 12 gm. of NaCl per day as estimated from urinary excretion of chloride while salt intake and output were presumed to be in balance. When the pellets were nearing the final stages of dissolution weakness and anorexia sometimes appeared. These symptoms were temporarily controlled with extra salt until the time when it was deemed advisable to insert new pellets. In several instances recently, new pellets were implanted at the end of 9 or 10 months without removal of the old ones. Signs of overdosage did not appear.

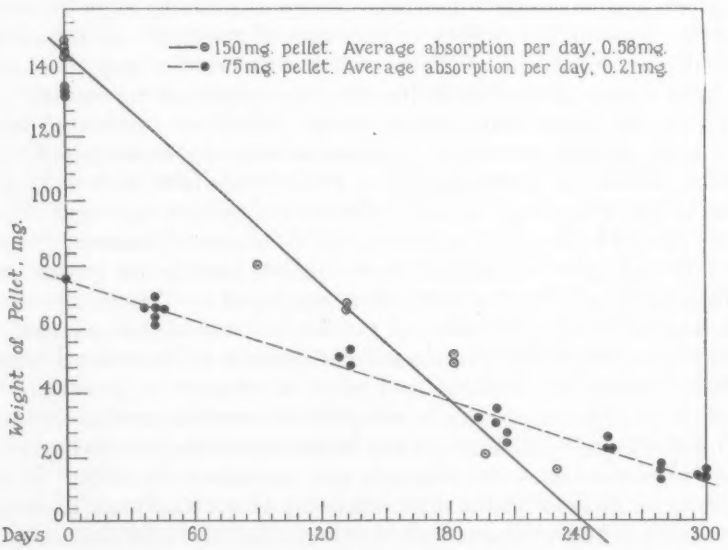

FIG. 1.—Rate of absorption of pellets.

Rate of Absorption of Pellets. If the weight of the pellets, after variable periods of absorption, is plotted against the time elapsing after implantation one obtains a distribution which tends to follow a straight line (Fig. 1). This indicates that the daily rate of absorption tends to be constant and that it does not decrease materially even after a pellet has become greatly reduced in size. This most fortunate

characteristic of pellet dissolution in both patients and animals has been observed previously not only with desoxycorticosterone but with other hormones as well,[4,6,26] and may probably be ascribed to a progressive roughening of the surface of the pellet along with an increase in permeability. In Figure 1 it may be seen that the daily rate of absorption of the large pellet averages 0.58 mg., and the maximum effective life is about 7 months. The daily absorption from the small pellet averages 0.21 mg. and the effective life may be estimated at 9 to 10 months. The rather small scatter of the individual points from the line representing the average rate of decline in pellet size indicates a reasonably constant rate of absorption from patient to patient and pellet to pellet. The effective life of a pellet is influenced by its weight, surface area, density and shape. By proper adjustments of these variables the maximum life of 10 months for a pellet might be materially lengthened. That pellet weight alone is not the sole factor is evidenced by the observation that the pellet weighing 150 mg. approaches complete absorption sooner than the one which weighs 75 mg.

TABLE 2.—SUMMARY OF THE EFFECTS OF PELLET THERAPY

	Case 1 (A. G.)	Case 2 (F. G.)	Case 3 (G. H.)	Case 4* (G. L.)	Case 5 (J. B.)	Case 6* (M. W.)	Case 7* (R. P.)
Duration of pellet treatment (mos.)	40	13	4	26	15	11	7
Clinical improvement	Excellent	Poor (death)	Poor (death)	Good	Excellent§	Good	Excellent
Overdosage symptoms	No	Yes	No	No	No	No	No
Hypoglycemia	Yes	Yes	Yes	No	Probably	No	No
Hematocrit:							
Before treatment	...	...	45	...	42	35	...
After implant	33	32	30	38	34	33	38
Blood urea nitrogen:							
Before treatment	36	17	47	10	20	12	...
After implant	10	10	15	9	6	15	12
Plasma chlorides (m.eq.):							
Before treatment	70	96	99	103	93	99	103
After implant	100	103	102	108	102	105	102
Blood pressure:							
Before treatment	85/60	100/60	78/50	110/70	75/50	90/60	105/70
After implant	105/70	110/60	98/60	115/70	90/50	105/65	115/75
Heart size:							
Before treatment	0.39	0.40	0.39	0.33	0.32	0.38	0.39
	−13	−14	−12	−27	−15	−3	−1
After implant	0.42†	0.48	0.40	0.39	0.37	0.39	0.40
	−2‡	−4	−8	−13	−8	+4	+1
Weight gain on treatment (lb.)	6	7	1	8	12	6	9
Number of 75 mg. pellets considered adequate for maintenance	4	.,.	...	6	6	4	4

* On salt therapy when first seen.
† Cardiothoracic ratio.
‡ Heart diameter as % of normal (Ungerleider and Gubner[31]).
§ Died after an appendectomy.

Clinical Results. Four of the 7 patients are living and in all cases the symptoms of the disease are under reasonably good control. One patient is employed as an animal caretaker, 2 are doing factory work, and 1 assists with housework in her home. Three patients have died. Two of the fatal cases could not be controlled by pellets or by injections of desoxycorticosterone acetate in oil. The 3d had been well controlled by pellets but died recently after an appendectomy. Some of the specific therapeutic results which were observed in the various patients are listed in Table 2. The various values which were obtained after treatment are expressed as the averages of several determinations

taken while the patients were under the influence of a dose of hormone which seemed to be optimum. The hematocrit was consistently low in all patients after pellet therapy had been started. The values, in fact, were distinctly lower than normal. It is highly probable that this depression in cell volume was due to anemia rather than to excessive hemodilution. Blood urea nitrogen remained low in all cases except in Case 3, where it rose just before death. Plasma chlorides and blood pressure were easily maintained in all patients. The cardiothoracic ratio rose in all cases. Inasmuch as the cardiac diameter of the patients before treatment tended to be distinctly smaller than the normal average, this rise was not due to abnormal dilatation. In Case 2, however, during a period when signs of overdosage were present, cardiac enlargement was well enough marked to be considered abnormal (see case report below). McGavack has advocated that treatment be regulated by observing changes in cardiac size.[19] All patients slowly gained weight. In Case 3 this was minimal, however, and in Case 2 there was an eventual decline even in the presence of edema. Patient No. 1 has shown an appreciable diminution in the pigmentation of his skin and mucous membrane. The others have shown no change.

Case Studies. CASE 1 (A. G.). This patient was first admitted in 1938 because of an asthenia which was so profound that he appeared almost completely paralyzed. Buccal pigmentation and the usual symptoms of Addison's disease were noted. After an infusion of 0.9% sodium chloride there was dramatic improvement and subsequently he was maintained in fairly good condition with a total daily salt intake of 20 gm. On January 30, 1940, one 150 mg. pellet of desoxycorticosterone acetate was implanted in the left subscapular region. Urine analysis revealed that his voluntary salt intake with food was 10 gm. per day. Within 4 to 5 months the left breast became enlarged and tender and upon palpation a well defined circular mass of tissue measuring 3 to 4 cm. in diameter could be made out just underneath the region of the nipple. A new pellet of similar size was inserted in the right subscapular region on August 14, 1940. The breast enlargement gradually subsided and has never been noticed again in this patient or in any others in the series. On April 4, 1941, what was left of the old pellets was replaced with two 75 mg. pellets. Two months later the patient was admitted because of a sore throat and vomiting. For 24 hours before admission the food intake had been almost nil. There were symptoms of hypoglycemia and blood sugar was 51 mg. per 100 cc. The signs of hypoglycemia promptly disappeared after administration of glucose intravenously and within a few days when the infection had subsided he was again asymptomatic. In September, 1941, he obtained a job in the animal room of the hospital, which he has held until the present time.

In June, 1942, after a Saturday morning's work he went out for a few "beers," ate no lunch, and after arriving home in the early evening, went to bed without eating. The next morning he was found unconscious on the floor of his bedroom. On admission to the hospital he was comatose but restless. The skin was cool and moist, blood pressure was 110/90, temperature 34.6° C., pulse 60. Unfortunately, no blood was taken for analysis, but his recovery of consciousness a few minutes after administration of glucose intravenously is highly suggestive of another episode of hypoglycemia. There was a sharp rise of temperature to 40° C. and patchy consolidation was discovered in one lung. He received 100 cc. of cortical extract and 20 mg. of desoxycorticosterone acetate in oil during the next 24 hours and after continuous vigorous treatment he completely recovered. The pneumonia was considered to be of

the "virus" type. With four 75 mg. pellets implanted he is now back at work with no symptoms whatever.

CASE 2 (F. G.). A 62-year old woman showing typical signs of Addison's disease was admitted in April, 1940. A chloride excretion test could not be completed because of extreme weakness, nausea, and a fall in plasma chlorides to 85 m.eq. Two pellets weighing 135 mg. each were implanted and she was discharged on a total dietary salt intake of approximately 10 gm. per day. Her weight on discharge was 110 pounds. After a month at home she reported that her strength and appetite were still poor although she had gained 3 pounds in weight. Blood pressure was 105/60, plasma chlorides 103, and blood urea nitrogen 9.8. Salt intake was increased to a total of 15 gm. per day. Two weeks later she awoke in the morning with edema of the hands, face and feet, orthopnea, dyspnea, cough and dilated neck veins. Her blood pressure was 135/70. All symptoms of overdosage disappeared within 24 hours after she was deprived of salt. With a total of 10 gm. of salt in the diet she remained weak and her appetite was poor. Her weight rose to 117 pounds and there was continual slight pitting edema of the ankles. She now complained of pain in various muscles and joints. Reduction of daily salt intake to a total of 5 gm. was followed by disappearance of the edema and fall of blood pressure to 115/60, but there was no change whatever in her subjective symptoms. In September, 1940, a generalized vesicular eruption accompanied by fever appeared and persisted for 1 week. Anorexia was now accompanied by spells of nausea and vomiting. In October, 1940, it was decided to increase her hormone dosage and markedly restrict her salt intake in the hope that physiologic effects of the hormone other than those controlling electrolytes might be brought into prominence. Four 150 mg. pellets were therefore implanted and the only source of salt allowed was that in her unsalted food—probably 3 gm. a day of NaCl. Slight edema of the face and ankles appeared but her weight fell to 110 pounds. Blood pressure gradually rose to 135/75 and in January, 1941, she began to experience substernal distress on exertion. The cardiac silhouette had increased to an abnormal size so that the cardio-thoracic ratio now measured 0.53. The electrocardiogram, which had previously been normal, now showed a left axis deviation and prolonged Q–T interval. There was no deviation of the S–T segment during exercise. After removal of one pellet the anginoid symptoms disappeared, but slight edema persisted. Potassium citrate, 3 gm. a day, caused no improvement or loss of edema but simply aggravated her feeling of weakness. In March, 1941, there was an episode of pleural effusion, pleural pain, and fever of 39.5° C. All dependent edema disappeared. Blood pressure was 145/80. The cardiac silhouette was extremely large but it was not certain whether this was due to pericardial effusion or cardiac dilatation. After 10 days the pleural process cleared and the heart shadow became normal. The pleural fluid produced no lesions when injected into guinea pigs. Two more pellets were removed and she was sent home with no therapy other than 15 gm. total salt per day. Her appetite grew worse, she became even weaker, there were severe muscle and joint pains and diarrhea. In May, 1941, desoxycorticosterone, 5 mg. a day, intramuscularly, was started. Her appetite failed entirely so that only a glass of milk was taken daily. One week after the start of injections she was seen at home in a semicomatose condition. The skin was cold and sweaty. Blood pressure was 110/60. There was no edema. A blood sample showed a urea nitrogen of 5.7 mg. per 100 cc., plasma chlorides 111 m.eq., hematocrit 26%, and blood sugar "too low to read." Death occurred several hours later in a convulsion. Autopsy showed severe bilateral atrophy of the adrenal cortex.

This patient did poorly on a wide range of dosage of desoxycorticosterone. Strength, appetite, and body weight were not maintained. Overdosage effects were produced at higher dose levels. Although plasma chlorides, blood urea nitrogen, and blood pressure were normal shortly before death, the hormone failed to maintain the blood sugar

level when food intake had declined, and death ensued from hypoglycemia.

CASE 3 (G. H.). A 47-year old colored woman was admitted in February, 1941, with the usual signs and symptoms of the disease. In March, 1941, two 75 mg. pellets were implanted. It was determined that the diet which she consumed contained a total of 5 to 8 gm. of salt per day. She became somewhat stronger, her appetite improved, but her weight remained unchanged. In July, 1941, she gradually lost 8 pounds of weight, grew weak, and lost her appetite. At this time she was running a fever of 38° C., blood pressure was 108/60, hematocrit 28%, plasma chlorides 98 m.eq., and blood urea nitrogen 15. Her salt intake was increased to a total of 15 to 20 gm. a day. There was no improvement and she stopped eating entirely. Blood sugar was 44 mg. on admission to the hospital at this time, chlorides 95 m.eq., blood urea nitrogen 23 mg. per 100 cc., hematocrit 26%, blood pressure 105/70. She was treated with parenteral glucose, saline and 10 mg. desoxycorticosterone daily. Nausea and vomiting grew more severe, the neck became stiff, and a Babinski sign was demonstrable on the left. Her temperature rose to 41.2° C. and coma ensued. Shortly before death her blood pressure was 120/70, plasma chlorides 110 m.eq., blood sugar 133 mg. per 100 cc., and blood urea nitrogen 32.3. Autopsy revealed bilateral tuberculosis of the adrenal cortex and a few microscopic tubercles of the periaortic lymph nodes, liver, and lungs. The brain and meninges were normal.

Although microscopic lesions were demonstrable in several organs of this patient in addition to the involvement of the adrenal cortex, it is unlikely that tuberculosis was the cause of the high fever and the fatal outcome. Hyperpyrexia has been observed in cases of uncomplicated Addison's disease by Loeb.[15]

CASE 4 (G. L.). This 35 year old woman was admitted in April, 1941, with a 2 year history of the disease. During the preceding year she had received extra salt and some injections of desoxycorticosterone acetate in oil. Four 75 mg. pellets were implanted and the total salt ingested with the diet was 5 to 7 gm. per day. The number of pellets was later increased to 6. She has had no nausea and her strength is sufficient to do a full day of housework. Her physical endurance, however, is not quite equal to that which she enjoyed before the onset of her disease. Although she has gained in weight there has been no edema.

CASE 5 (J. B.). A 28 year old man admitted in January, 1942, complained of symptoms of the disease for at least 2 years. A chloride excretion test could not be completed because of nausea, vomiting, and a generally poor clinical condition which was precipitated by the test. With four, and later six 75 mg. pellets in place and a total daily dietary NaCl intake of 10 to 12 gm. he gained weight and was able to do hard physical work. There was an episode associated with a respiratory infection when he failed to eat for 24 hours and was found unconscious in bed in the morning. It may be presumed that the coma was due to hypoglycemia, inasmuch as recovery was complete within a few hours after a generous intake of fruit juices. Recently he entered the hospital with typical signs of appendicitis. Symptoms had been present for 48 hours. During the next 8 hours he was prepared with glucose, saline, 5 mg. of desoxycorticosterone, and 30 cc. of cortical extract, after which a gangrenous appendix was removed. During the first postoperative day he received 94 cc. of cortical extract along with flucose and saline. His temperature reached 40.7° C. but blood pressure never fell below 110/70. By the third postoperative day his condition seemed excellent. He was taking food by mouth and no parenteral fluids were administered. On this day 28 cc. of extract was given. He had received no desoxycorticosterone since before the operation. When seen at 5:15 A.M. by the nurse he seemed to be in excellent condition except for slight

nausea. At 6 A.M. he was found dead. It was learned that he had refused his supper and that his last food had consisted of a glass of milk at 3 P.M. Autopsy revealed adrenal cortical atrophy so severe that nothing unquestionably identifiable as cortical tissue could be demonstrated. The heart was somewhat dilated.

This case is an example of the relative ease with which certain patients may be maintained with pellets during the absence of stress but who present a difficult problem in the presence of intercurrent illness. The cause of death may have been hypoglycemia, but this is conjectural.

CASE 6 (M. W.). A 30 year old woman who had suffered from the disease for 9 months was admitted in April, 1942. After the insertion of four 75 mg. pellets she was sent home on a diet containing 6 to 8 gm. of NaCl per day. She is able to do factory work at present and considers her strength to be adequate although not quite up to her previous normal.

CASE 7 (R. P.). A 33 year old man, admitted in September, 1942, gave a 9 months history of Addison's disease. With four 75 mg. pellets in place he is now back at work as foreman in a steel mill and feels in every way as well as he had before the onset of his disease. His daily dietary salt intake totals approximately 10 gm.

Comments. It is important to note that it was evident very early in the treatment of the 2 patients who were poorly maintained by pellets that they were showing a poor response. Those patients whose initial response was good have continued to maintain a favorable course, and, except for complications due to intercurrent illness, they have not shown any important fluctuations in status.

The results of treatment in Case 2 may be considered totally unsatisfactory. Desoxycorticosterone therapy failed to maintain the appetite and body weight, and was not followed by a subjective feeling of improvement. It also failed to prevent a terminal hypoglycemia induced by fasting. In Case 3 the maintenance dose of hormone was probably too low, but more intensive treatment with desoxycorticosterone by injection failed to alleviate the severe gastric symptoms, the hyperpyrexia, and the coma which preceded death. It is noteworthy, however, that both of these patients died without going into the classical "crisis" of Addison's disease. Blood pressure did not fall and there was no evidence of depletion of electrolytes and fluid.

In the 5 remaining patients the pellet therapy may be considered to have produced very good results. All were able to work. One felt as well and as strong as he did before the onset of the disease, and the others not only felt reasonably well but were not seriously restricted in their activities. Patients Nos. 1 and 4, who had been maintained with salt therapy alone prior to treatment with pellets, felt stronger and in better general health while under the latter form of treatment.

The frequency with which hypoglycemia was encountered is notable. The present results add confirmation to previous reports that desoxycorticosterone does not repair the defect in carbohydrate metabolism which is associated with adrenal insufficiency. When desoxycorticosterone acetate is employed for maintenance of patients, one must, therefore, point out to the patient the danger of going without food.

It should be insisted that an adequate food intake be maintained even though the appetite fails. Hypoglycemic reactions are particularly apt to occur during infections and are probably attributable not only to an impaired appetite but also to the stress of the infection itself.

In the presence of a severe infection or any serious complicating illness the requirement for hormone is materially increased. The amount of hormone derived from the pellets becomes inadequate and intensive therapy with additional hormone is imperative. In such emergencies cortical extract in large doses should be given inasmuch as this preparation should theoretically provide cortical hormones with important physiologic properties not possessed by desoxycorticosterone. Additional desoxycorticosterone acetate in oil may also be given if necessary to assist in the maintenance of blood pressure, and electrolyte and water balance.

Summary and Conclusions. Five out of a series of 7 patients with Addison's disease were well maintained for 7 to 40 months by pellets of desoxycorticosterone acetate and were able to carry on work involving moderate physical activity. The other 2 patients were not satisfactorily controlled either by pellets or by the compound administered by injection. Both of these patients died from the disease. One patient died suddenly after an attack of appendicitis at a time when he seemed to be convalescing satisfactorily.

Patients under therapy with pellets of desoxycorticosterone acetate may easily be thrown into hypoglycemia by fasting. They are particularly vulnerable in the presence of an infection. Aside from its failure to restore normal carbohydrate metabolism, desoxycorticosterone is deficient in other respects. The symptoms of the disease cannot be controlled in all cases, and death cannot invariably be prevented no matter what dose is employed. It should be pointed out, however, that some cases of Addison's disease also do not respond to cortical extract.

On the basis of the present observations it is concluded that pellet therapy with desoxycorticosterone acetate is highly useful for the maintenance of most patients with Addison's disease. During infections or other conditions imposing stress the requirement for hormone is increased, and under these circumstances additional therapy, chiefly in the form of cortical extract, should be given.

The effective life of the 75 mg. pellet which is being used at present is approximately 9 to 10 months, the average daily absorption is 0.21 mg. per pellet, and the average number of pellets required is four to six. The rate of absorption is reasonably constant from patient to patient and pellet to pellet.

It has been possible to implant pellets without subjecting patients to a lengthy preliminary period of therapy with injections. In the one case in which removal of pellets was necessary because of overdosage the number of pellets had been deliberately increased for special reasons. Insertion of new pellets without removal of the old ones after the latter have been almost completely absorbed has not produced signs of overdosage.

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THE USE OF FIBRINOGEN IN A RAPID METHOD OF DETERMINING CELL VOLUME

By Seymour Gray, B.A., M.D.

INSTRUCTOR IN MEDICINE, THE UNIVERSITY OF CHICAGO
CHICAGO, ILL.

(From the Department of Medicine)

The determination of the volume of packed red cells has assumed an increasingly important rôle not only in the differential diagnosis of the anemias but in the evaluation of blood volume, shock, hemorrhage and dehydration. The volume of packed red blood cells "is an accurate index of the degree of anemia and is more easily and more accurately measured than even the red cell count. The cell volume constitutes the most useful single criterion of the degree of anemia available. It also serves to correct the sedimentation rate of blood for the influence of anemia or polycythemia."[10] The determination of the mean corpuscular volume, saturation index and volume index depends

in part upon the cell volume. The value of these studies in facilitating the diagnosis of the anemias has been established by Haden,[6] Osgood,[7] Wintrobe[10] and others. Finally, the cell volume determination has become an increasingly useful adjunct to the surgeon in recent years in measuring hydration and in the study of blood volume, hemorrhage and shock.

The volume of packed red cells may be determined by centrifuging a quantity of blood to which a suitable anticoagulant has been added. Centrifugation is carried out until the cell volume remains constant and no further packing occurs. The time of centrifugation has varied widely with different workers. It depends upon the type of hematocrit, the speed of centrifugation and the length of the arm of the centrifuge. The recommended period of centrifugation varies from 30 minutes at 3000 r.p.m.[10] to 90 minutes at the same speed.[4] Haden[6] suggests 60 minutes at 2500 r.p.m. while other workers centrifuge the blood for 90 minutes at 2400 r.p.m.[9] The volume of packed red cells determined by centrifugation for 30 to 90 minutes is accurate to within 0.5%. The object of this report is to describe a method of determining cell volume in 5 minutes with an accuracy equal to the 30-to-90 minute method.

Fahraeus[3] in 1921 established the foundation for our present knowledge of the suspension stability of blood. He noted that fibrinogen decreased the negative charge on the red blood cells thus causing increased rouleaux formation and found that the increased sinking velocity of the red blood cells depended upon this increased agglutination or aggregation of the red blood cells. Maximum aggregation and the most rapid sedimentation of the red blood cells occurred in solutions of fibrinogen.

The rapidity of sedimentation depends upon the size of the cell aggregates which in turn depends upon the fibrinogen content of the solution in which the red blood cells are suspended. The physicochemical properties of fibrinogen affect the red blood cells in such a manner that the greater the plasma fibrinogen content the greater is the size of the settling red cell aggregates. The rate of settling of the red blood cells is directly proportional to the square of the radius of the suspended particles and follows Stokes' law of hydrodynamics. The size of the cell aggregates is thus the critical factor in the rate of settling of the red blood cells.

We have observed in a previous communication[5] that the addition of electrophoretically pure fibrinogen to heparinized blood increased the sedimentation rate 300 to 800%, the increase being proportional to the amount of fibrinogen added. When 0.4 gm. per 100 cc. fibrinogen was added to whole blood and the sedimentation rate was determined by Wintrobe's method,[10] the cells settled so rapidly that they approached cell volume at the end of 1 hour. Since the rate of settling is proportional to the size of the cell aggregates and since the size of the cell aggregates depends upon the amount of fibrinogen added to the plasma, we investigated the use of fibrinogen in developing a rapid method for determining cell volume.

Method. Electrophoretically pure bovine fibrinogen, in the salt form prepared at the Armour Laboratories by the methods developed at the Department of Physical Chemistry, Harvard Medical School and supplied at the suggestion of Dr. Edwin J. Cohn, was used in all experiments. A neutral isotonic solution of fibrinogen was prepared by dissolving 0.31 gm. of the salt in 10 cc. of distilled water. Since the fibrinogen content is approximately 52% and the salt content 48% the final protein concentration of the solution was 1.6 gm. per 100 cc.

Heparin was the anticoagulant employed in all studies; 0.25 cc. of the fibrinogen solution (equivalent to approximately 4 mg. of pure fibrinogen) was added to 1 cc. of heparinized blood. The blood now containing an additional fibrinogen content of 0.4 gm. per 100 cc. was shaken gently to insure uniform mixing and was placed in a Wintrobe hematocrit tube and centrifuged for exactly 5 minutes. An interval timer was used to control accurately this period of centrifugation. Cell volumes were determined by reading the level of the cells and the upper level of the clear plasma in the hematocrit at the end of exactly 5 minutes of centrifugation. Determinations were made at 1500 r.p.m., 1800 r.p.m. and 2100 r.p.m. with an International Centrifuge Size 1, Type C, radius 17 cm.

Control studies were made simultaneously with heparinized blood containing no added fibrinogen and with blood containing an additional 0.25 cc. of homologous plasma or saline.

The cell volumes determined at the end of 5 minutes of centrifugation were compared with the cell volumes of the unaltered heparinized blood centrifuged for 60 minutes.

Sedimentation rates of the control blood and the blood with the added fibrinogen, plasma or saline were determined in each instance by Wintrobe's method.[10]

Results. The 5-minute method of determining cell volume was applied to 50 patients whose cell volumes varied between 17 and 68%. The cell volume determinations were between 20 to 30% in 6 of 50 patients, 30 to 40% in 10, 40 to 50% in 23, 50 to 60% in 7 and 60 to 70% in 4. The uncorrected sedimentation rates of the unaltered blood specimens varied between 0.5 and 64 mm. at the end of 1 hour (Table 1).

TABLE 1.—CELL VOLUMES OF BLOOD CENTRIFUGED FOR 60 MINUTES COMPARED WITH BLOOD CONTAINING ADDED FIBRINOGEN CENTRIFUGED FOR 5 MINUTES AT 1800 R.P.M.

Pt.	Cell volume		Sedimentation rate mm. in 1 hr.
	Control blood centrifuged 60 min.	Control blood + 0.4 gm. per 100 cc. fibrinogen centrifuged 5 min.	
K	17.0	16.7	
B	26.0	26.2	18.0
D	29.0	29.0	34.0
H	29.5	29.8	18.0
C	33.2	32.8	33.0
A	37.5	37.1	41.0
X	43.2	43.2	9.0
I	43.3	42.9	5.0
Z	44.8	45.0	17.0
J	46.5	46.5	42.0
Y	48.0	48.0	5.0
F	49.5	50.0	11.0
R	58.0	57.5	.5
E	58.1	58.0	2.0
G	67.0	67.0	.5
P	68.0	67.5	.5

Centrifugation for 5 minutes of blood containing the added solution of fibrinogen yielded cell volume determinations which were accurate to within 0.5% of the cell volumes of the unaltered blood centrifuged for 60 minutes at 1800 r.p.m. (Table 1). The mean deviation of the cell volumes obtained in 5 minutes was 0.24% in the 50 cases studied.

The accuracy of the 5-minute method is thus within the range of accuracy of the present methods requiring 30 to 90 minutes of centrifugation.

Table 1 demonstrates the accuracy of the method within a wide range of cell volumes (17 to 68%) and sedimentation rates (0.5 to 42 mm.). That this accuracy is independent of cell volume range or sedimentation rate is also illustrated in Figure 1 which compares the cell volumes of blood centrifuged for 60 minutes with blood containing added 0.4 gm. per 100 cc. fibrinogen centrifuged for 5 minutes at widely divergent cell volumes and sedimentation rates.

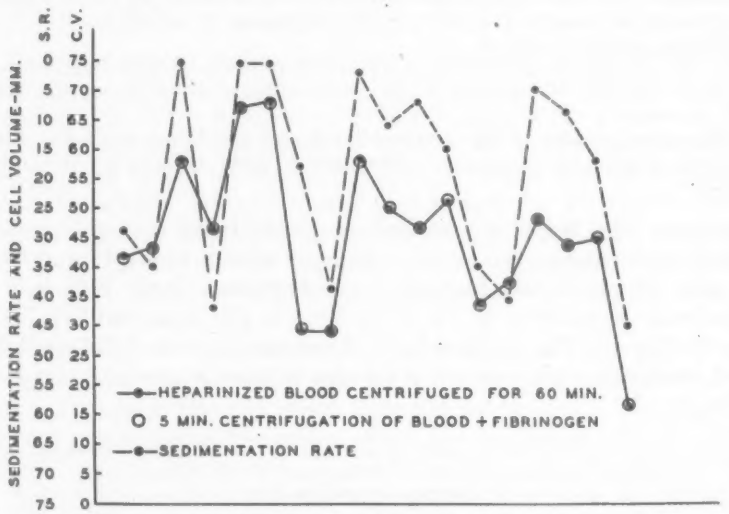

FIG. 1.—Comparative cell volume of blood centrifuged 60 minutes and blood with added fibrinogen centrifuged for 5 minutes.

The effect of the speed of centrifugation upon the 5-minute cell volume determinations is noted in Table 2. The most accurate results were obtained by centrifuging the blood at 1500 to 1800 r.p.m., the mean deviation from the 60-minute control being 0.26% and 0.17% respectively.

The 5-minute cell volume was identical with the 60-minute control in 4 of the 12 studies at both 1500 and 1800 r.p.m. The greatest single deviation was 0.5%. At 2100 r.p.m. the dilution effect became apparent and the 5-minute cell volumes were 1 to 2% lower than the 60-minute determinations. The mean deviation at 2100 r.p.m. was 1.25%. It may be concluded therefore that centrifugation should be carried out at 1500 to 1800 r.p.m. and that an error is introduced at higher speeds of centrifugation. Table 2 also demonstrates that the

accuracy of the 5-minute method is not affected by the range of cell volume or sedimentation rate.

TABLE 2.—EFFECT OF SPEED OF CENTRIFUGATION ON CELL VOLUMES DETERMINED IN 5 MINUTES

Control blood centrifuged 60 min. at 1800 r.p.m.	Control blood with added fibrinogen centrifuged for 5 min.			Sedimentation rate of control blood mm. in 1 hr.	Sedimentation rate of control blood with added fibrinogen mm. in 1 hr.
	1500 r.p.m.	1800 r.p.m.	2100 r.p.m.		
46.9	47.2	46.8	45.2	25.0	48
33.2	33.2	33.0	32.5	31.0	61
32.2	32.3	31.9	31.5	60.0	67
22.8	22.8	22.6	22.2	46.0	73
52.0	52.0	51.7	50.5	18.0	53
36.5	36.2	36.0	35.5	12.0	55
44.0	44.3	43.8	42.7	10.0	47
22.8	22.8	22.6	21.8	64.0	76
63.0	63.2	63.0	61.5	1.0	33
67.5	68.0	67.5	66.0	0.5	22
69.0	69.5	69.0	67.5	0.5	14
44.0	44.5	44.0	42.8	19.0	47

Comparative 5-minute cell volume determinations of blood containing added fibrinogen, homologous plasma and saline indicate that accurate and consistent results are obtained with fibrinogen only (Table 3). This was demonstrated in the following manner: 0.25 cc. of the fibrinogen solution, homologous plasma and physiologic saline was added respectively to each of 3 tubes containing 1 cc. of blood, and the cell volume was determined by centrifugation for 5 minutes in the usual manner. Consistent and accurate cell volume determinations were obtained only in the blood specimens containing the added fibrinogen solution. The mean deviation from the 60-minute control was 0.2%, and the maximum deviation for this group was 0.5% (Table 3).

TABLE 3.—COMPARATIVE 5-MINUTE CELL VOLUMES OF BLOOD CONTAINING ADDED FIBRINOGEN, HOMOLOGOUS PLASMA, AND SALINE

Control blood centrifuged 60 min. at 1800 r.p.m.	1 cc. blood + 0.25 cc. fibrinogen solution centrifuged for 5 min.		1 cc. blood + 0.25 cc. homologous plasma centrifuged for 5 min.		1 cc. blood + 0.25 cc. physiologic saline centrifuged for 5 min.
Cell volume	1500 r.p.m.	1800 r.p.m.	1500 r.p.m.	1800 r.p.m.	1800 r.p.m.
22.8	22.8	22.6	21.8	20.4	25
32.2	32.3	31.9	30.5	28.5	35
33.2	33.2	33.0	35.0	34.2	36
36.5	36.2	36.0	36.7	36.0	40
44.0	44.5	44.0	48.0	42.2	49
46.9	47.2	46.8	48.5	46.7	51
47.0	..	47.2	..	51.5	53
52.0	52.0	51.5	55.5	50.0	57
63.0	63.2	63.0	67.5	65.0	68

Dilution of the blood with homologous plasma gave widely variable results. Centrifugation at 1500 r.p.m. for 5 minutes yielded cell volume determinations 0.2 to 4.5% higher than the 60-minute cell volumes in some cases and 1 to 1.7% lower in others. The mean devition from the control was 2.3% with a maximum deviation of 4.5%. Centrifugation at 1800 r.p.m. yielded cell volume determinations 0.2

to 2.4% lower than the 60-minute control in some instances and 1 to 4.5% higher in others. The mean deviation was 2.1% and the maximum deviation 4.5%.

The addition of physiologic saline to blood resulted in consistently high cell volumes when the blood was centrifuged for 5 minutes at 1800 r.p.m. The mean deviation was 4.2% when compared with the 60-minute control, and the maximum deviation was 6%.

When unaltered heparinized blood was subjected to consecutive 5-minute centrifugations for 1 hour at 1800 r.p.m. (Fig. 2) the cell volume reading at the end of the first 5 minutes was 60.8% and then fell to 50.8, 49.5, 48.5, and 47.5% at 10, 15, 20 and 25 minutes respec-

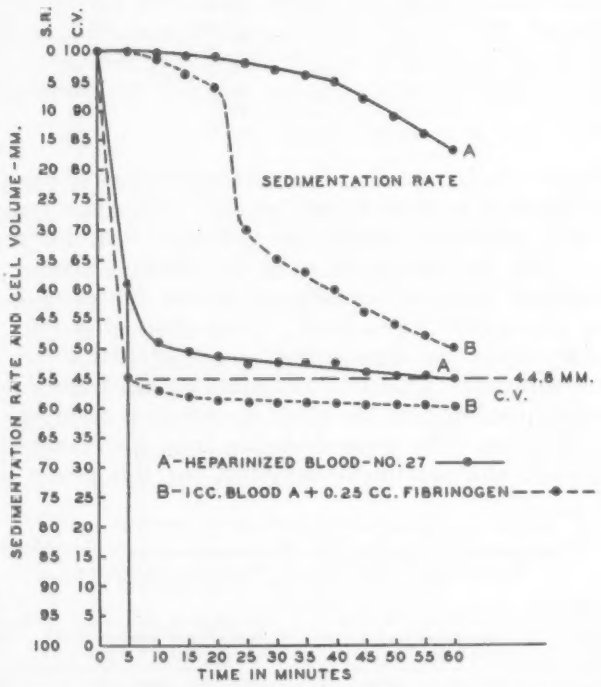

FIG. 2.—The effect of fibrinogen upon the sedimentation rate and cell volume of blood.

tively until complete packing occurred at 60 minutes and a constant reading of 44.8% was obtained. When 1 cc. of the same blood containing an added 0.25 cc. of fibrinogen solution was centrifuged for 5 minutes at 1800 r.p.m. the cell volume was 45%, almost identical with the cell volume determination of 44.8% observed in the unaltered blood centrifuged for 60 minutes (Fig. 2). During the subsequent 5-minute periods of centrifugation the cell volume fell to 43, 42, 41.2 and 40.9% at 10, 15, 20 and 25 minutes respectively as a result of dilution with the fibrinogen solution. The sedimentation rate was increased from 17 to 50 mm. at the end of 1 hour by the addition of 0.25 cc. of the fibrinogen solution to 1 cc. of blood (Fig. 2).

Figure 3 demonstrates the effect of fibrinogen upon blood with a

slow sedimentation rate. The addition of 0.25 cc. of the fibrinogen solution to 1 cc. of this blood increased the sedimentation rate from 5 to 49 mm. in 1 hour.

After 5 minutes of centrifugation at 1800 r.p.m. the cell volume reading of the control blood specimen was 67% compared with a determination of 48% for the same blood containing the added fibrinogen centrifuged for the same period of time at the same speed. At the end of 60 minutes of centrifugation, however, the control cell volume was 48%, identical with the 5-minute determination of the blood containing added fibrinogen (Fig. 3).

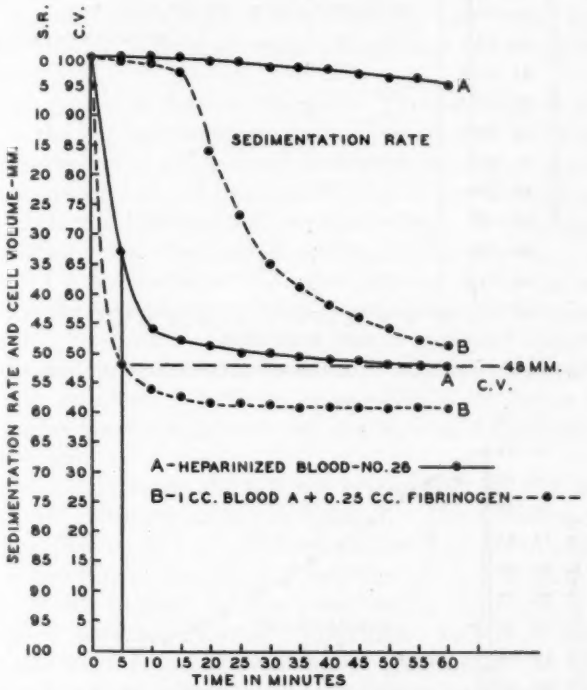

FIG. 3.—The effect of fibrinogen upon the sedimentation rate and cell volume of blood.

The 5-minute method of determining cell volume is equally accurate for blood with a high cell volume and slow sedimentation rate as demonstrated in Figure 4. Although the sedimentation rate of the blood was increased from the original rate of 0.5 mm. to only 5 mm. in 1 hour by the addition of fibrinogen, the 5-minute centrifugation of this blood yielded a cell volume of 67%, identical with the cell volume obtained by centrifuging the control blood for 60 minutes at 1800 r.p.m. The cell volume reading of the control blood after 5 minutes of centrifugation was 86.5% (Fig. 4). Centrifugation of the unaltered blood for 45 minutes at 3000 r.p.m. also yielded a cell volume reading of 67%, again confirming the accuracy of the 5-minute determination.

The accuracy of the method is not affected by low cell volumes or

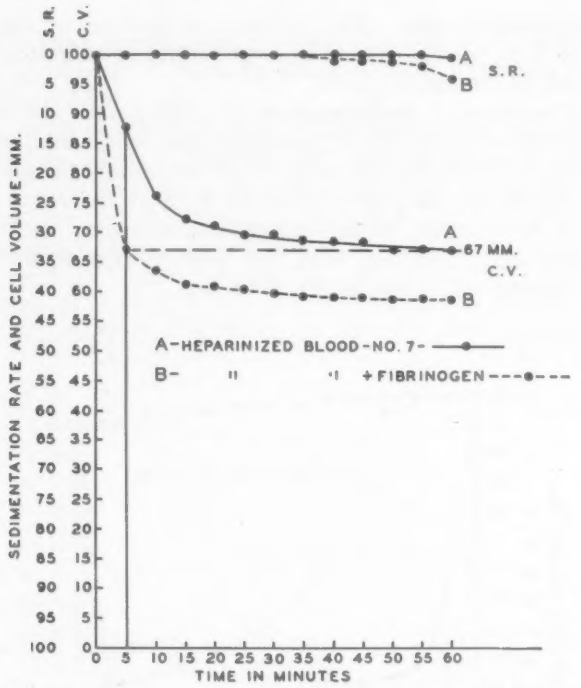

FIG. 4.—The effect of fibrinogen upon the sedimentation rate and cell volume of blood.

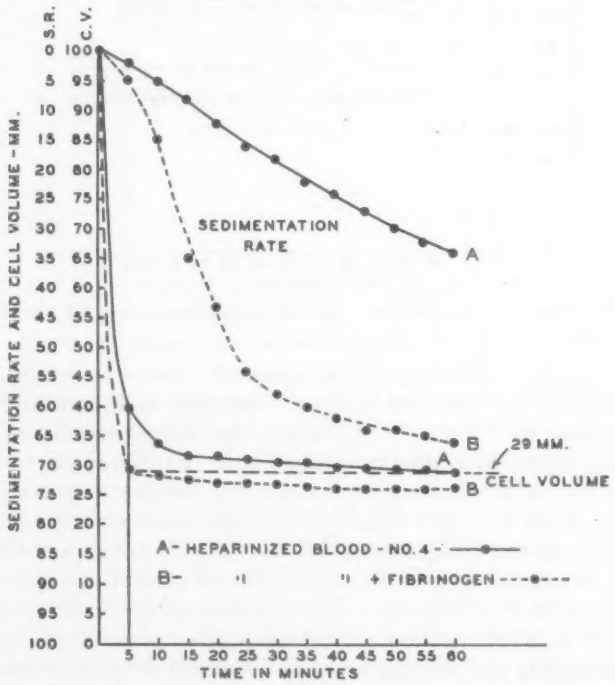

FIG. 5.—The effect of fibrinogen upon the sedimentation rate and cell volume of blood.

high sedimentation rates. This is demonstrated in Figure 5. The cell volume of the blood containing added fibrinogen was 29% after 5 minutes of centrifugation at 1800 r.p.m. The unaltered blood yielded cell volume readings of 39.8% after 5 minutes of centrifugation and 29% after 60 minutes of centrifugation, the latter identical to the 5-minute cell volume of the blood containing the added fibrinogen. The sedimentation rate of the control blood was 34 mm. in 1 hour and was increased to 66 mm. by the addition of the fibrinogen solution.

Discussion. There are two factors responsible for this rapid method of determining cell volume. The primary factor is the fibrinogen itself. The addition of an excess of fibrinogen to blood increases the size of the red cell aggregates thus promoting very rapid settling so that the cell volume is reached within 5 minutes of centrifugation at 1500 to 1800 r.p.m.

The second factor is that of dilution. The addition of 0.004 gm. fibrinogen in the dry salt form to 1 cc. of blood does not result in as accurate and consistent cell volume determinations after 5 minutes of centrifugation as does the addition of 0.25 cc. of the fibrinogen solution containing 0.004 gm. fibrinogen as recommended. It has been repeatedly demonstrated that the rate of settling of blood cells is increased by dilution. Cutler[2] believes that the dilution factor increases the rate at which the red blood cells fall because the "activating factors in the plasma," presumably fibrinogen, "have a greater opportunity to drive the remaining cells into rouleaux." Others[8] believe that by decreasing the number of particles in suspension the mean free path of such particles becomes greater, and the rate at which the particles settle is increased.

That the dilution factor alone is not responsible for this method of determining cell volume is shown in Table 3. The addition of 0.25 cc. of homologous plasma to 1 cc. of blood yielded inaccurate and variable cell volume determinations after 5 minutes of centrifugation. This may be explained, in part, by the variation in fibrinogen content of different blood plasmas. Grossly inaccurate and high cell volume determinations were noted after a similar dilution of the blood with physiologic saline since saline inhibits the aggregation of red blood cells.

If heparinized blood is subjected to consecutive 5-minute centrifugations for 60 minutes at 1800 r.p.m. and the level of the red blood cells is recorded graphically, the resulting figure is a vertical curve consisting of two phases (Figs. 2, 3, 4 and 5). The first phase is that in which the red blood cells descend very rapidly in the tube as a result of centrifugation; this phase is of 10 minutes' duration and is graphically represented by a straight line which may be vertical or slightly diagonal, depending upon the size of the cell aggregates. The second phase is that of packing and is influenced by the degree of anemia or dilution. This phase is complete when the level of red blood cells remains constant after several consecutive 5-minute periods of centrifugation. At 1800 r.p.m. this phase requires 40 to 50 minutes of centrifugation. At this point the cell volume is attained.

When the fibrinogen solution is added to the blood rapid aggregation

of the red blood cells occurs and large cell aggregates are formed. Since the rate of settling depends upon the size of the cell aggregates the red blood cells fall very rapidly upon centrifugation at 1500 to 1800 r.p.m. The curve becomes more vertical and is shifted to the left so that the first phase is complete in 5 minutes (Figs. 2, 3, 4 and 5). This period of rapid settling determines the cell volume of the blood. The cells have fallen so rapidly that the cell volume determination after 5 minutes of centrifugation is the same as that of the unaltered blood after 60 minutes. Centrifugation of this blood containing the added fibrinogen solution for more than 5 minutes produces the onset of the second phase. Since this second phase is influenced considerably by the degree of dilution further packing of the cells occurs, and the cell volume falls below that of the unaltered blood (Figs. 2, 3, 4 and 5). For this reason it is very important to time the 5-minute period of centrifugation accurately. If this is not done the cell volume determinations will be low as a result of the dilution factor. The use of an interval timer is the simplest means of insuring accuracy.

The phases of rapid settling and packing observed in blood subjected to consecutive 5-minute periods of centrifugation are very similar to the phases of sedimentation and packing described by Cutler[2] for rapidly sedimenting blood.

The pronounced accelerating effect of the fibrinogen solution upon the sedimentation rate of erythrocytes is demonstrated in Table 2 where increases in the sedimentation rate of 300% or more may be observed. If the sedimentation rates are recorded graphically vertical curves "indicating the most rapid form of sedimentation"[2] are observed when the fibrinogen solution is added to blood (Figs. 2, 3 and 5). All sedimentation rates in this communication are recorded directly without correcting for anemia or dilution since we are concerned primarily with the sedimentation rate of the blood containing the added fibrinogen solution in its relation to the rapid determination of cell volume rather than with the corrected sedimentation rate as a manifestation of disease. Although there is considerable controversy concerning the advisability of correcting for anemia, the addition of 0.25 cc. of homologous plasma to 1 cc. of blood does result in a moderate increase in the sedimentation rate, but the increase in rate does not approach that caused by the addition of 0.25 cc. of the fibrinogen solution to an equal amount of blood. Physiologic saline decreases the sedimentation rate. Neither homologous plasma nor saline is satisfactory for the rapid method of determining cell volume.

It should be emphasized that the time and dilution factors described have been standardized for the Wintrobe hematocrit and that this rapid method is accurate only when the Wintrobe hematocrit is used. The above principles, however, may be applied to any other hematocrit.

The average cell volume determination by the 5-minute method for 10 healthy men and women was 45.9 and 41.5% respectively, comparing favorably with the normal values of other investigators.[6,7,10]

The advantages of a rapid and accurate method of determining cell volume in the study of shock, hemorrhage and dehydration are obvious. The method of determining the volume of packed red cells in 5 minutes

as described is simple, does not require a high speed centrifuge and is as accurate as the present methods requiring 30 to 90 minutes of centrifugation.

Summary. 1. Centrifugation for 5 minutes of heparinized blood containing an electrophoretically pure bovine fibrinogen solution yielded cell volume determinations which were accurate to within 0.5% of the cell volumes of unaltered blood centrifuged for 60 minutes at 1800 r.p.m. The accuracy of the 5-minute method was within the range of accuracy of present methods requiring 30 to 90 minutes of centrifugation.

2. This rapid method of determining cell volume was applied to 50 patients and was found exceedingly accurate within a wide range of cell volumes (17 to 68%) and sedimentation rates (0.5 to 42 mm.). The mean deviation was 0.24% and the accuracy of the method was not affected by the range of cell volume or sedimentation rate.

3. Comparative 5-minute cell volume determinations of blood containing added fibrinogen, homologous plasma and saline indicated that accurate and consistent results were obtained only in the blood specimens containing the added fibrinogen solution.

4. The most accurate results were obtained by centrifugation of the blood containing the fibrinogen solution for 5 minutes at 1500 to 1800 r.p.m.

5. When heparinized blood was subjected to consecutive 5-minute periods of centrifugation and the level of the red blood cells was recorded graphically the resulting figure was a vertical curve consisting of two phases: rapid settling and packing. The addition of the fibrinogen solution to blood increased the rate of settling of the erythrocytes and produced a more vertical curve so that the first phase was completed in 5 minutes and recorded the cell volume.

6. The pronounced accelerating effect of the fibrinogen solution upon the sedimentation rate of erythrocytes and the mechanism of the rapid method of determining cell volume were discussed.

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THE INHALATORY ROUTE FOR PROPHYLAXIS AND TREATMENT OF EXPERIMENTAL INFLUENZA*

I. The Distribution of Inhaled Material

By the Personnel† of Naval Laboratory Research Unit No. 1
UNIVERSITY OF CALIFORNIA
AND
William R. Lyons, Ph.D.
DIVISION OF ANATOMY, UNIVERSITY OF CALIFORNIA, SCHOOL OF MEDICINE
BERKELEY, CALIF.

The work of Smorodintseff and co-workers[30,31] and Nachaev[18] on the prevention and treatment of human influenza by inhalation of immune horse serum has stimulated our interest in this subject. Complete data on the apparatus and methods used by the Russian authors are not given in their available publications.

The chief route of transmission of epidemic influenza is by direct inhalation of infective material either in the form of small droplets or droplet-nuclei of the Flügge type. Once infection has established itself it is probable that the whole respiratory tract from nasal epithelium to the distal alveoli of the lungs is involved. Nevertheless, uncomplicated influenza must still be regarded as a localized infection, since it is rare that other body sites are infected.

The approach of Smorodintseff and Nachaev in attempting to reach the susceptible or infected area with serum by the only direct route is a most logical one. Intravenous and intramuscular injections of immune serum were attempted during the epidemic of 1918–19 with doubtful results, while it has been repeatedly shown[13,32,39] that the intranasal administration of immune serum to mice is superior to subcutaneous, intra-abdominal or intravenous routes for protection and treatment of the experimental animal.

Before attempting clinical trials of the inhalation method it was decided to investigate the efficiency of the mode of administration in penetrating the deeper passages of the respiratory tract. There is no doubt that intranasal insufflation of fluids results in an uneven distribution of the material. The ideal method of administration to humans would be one which not only would distribute the inoculated material evenly but would reach the deeper respiratory passages as well. The

* The opinions advanced in this paper are those of the writers and do not represent the official views of the Navy Department.

† The Unit Personnel consists of Albert P. Krueger, Commander (M C) U. S. N. R., Officer-in-Charge, and

A. S. Browne, Lieut., U. S. N. R.
O. J. Golub, Lieut., U. S. N. R.
L. E. Rosenberg, Lieut., U. S. N. R.
N. S. West, Lieut., U. S. N. R.
A. H. Jacobs, Lieut. (jg.), (M. C.) U. S. N. R.
J. R. Mathews, Lieut. (jg.), U. S. N. R.
H. M. S. Watkins, Lieut. (jg.) U. S. N. R.
A. J. Glazko, Ensign, U. S. N. R.
G. B. Saviers, Ensign, U. S. N. R.
M. D. Thaxter, Ensign, U. S. N. R.
I. L. Shechmeister, Pharm., U. S. N. R.
W. L. Axelrod, C.Ph.M., U. S. N. R.
E. R. Chisholm, Ph.M., 1c, U. S. N. R.
P. J. Smith, Ph.M., 1c, U. S. N.
C. R. Webb, Jr., Ph.M., 1c, U. S. N. R.
H. R. Burkhead, Ph.M., 2c, U. S. N. R.
W. D. Won, Ph.M., 2c, U. S. N. R.
D. D. Mentz, Ph.M., 3c, U. S. N.
A. D. Dolan, Jr., H.A., 1c, U. S. N.

disadvantages of the intranasal route in mouse inoculations of both serum and virus suspensions led Zellat and Henle[39] to suggest atomization of serum in order to assure its more uniform distribution.

It is evident that any device to be used in inhalation experiments must be capable of producing a fog of very finely divided particles and, at the same time, operate at a high rate of efficiency in terms of amount of liquid atomized per unit volume of air required for the atomization process. In this paper, the first part of a series, is described the construction and preliminary performance data of an improved atomizer developed in this laboratory.

All atomizers operate on essentially the same principle, namely, impinging air and liquid at sufficient velocity to break up the liquid into small particles. The ordinary household fly-sprayer is a good example of a simple atomizer employing a relatively slow moving stream of air directed at right angles across a liquid source rising in a capillary tube. Several refinements have been made in this basic type atomizer. A modified Wells atomizer[38] built into a Florence flask and employing a mixture of liquid and air driven at high velocity through a small orifice has given good results. Such atomizers have the advantage of delivering a uniformly small particle size by screening out the larger particles within the body of the flask. These droplets are returned to the reservoir and then re-atomized. This type of atomizer has the disadvantage of a very low total output per unit time, and, for work of the nature being done in this laboratory, is quite unsuitable.

The atomizer herein described delivers uniformly small particles in an efficient manner at the rate of approximately 55 to 65 cc. per hour and yet operates within a pressure range readily obtainable in the laboratory. Figure 1 shows the atomizer built for these studies; it is a composite modification of the existing atomizers of this type rather than something entirely new.

The body of the atomizer is a 2 liter Florence flask. The jet, *A*, is made of $\frac{1}{4}$ inch (I.D.) glass tubing drawn to a tip with an orifice of approximately $\frac{1}{16}$ inch. Within this tube is fused a smaller tube of $\frac{1}{16}$ inch (I.D.) drawn to a tip of approximately $\frac{1}{32}$ inch orifice. The air stream passes up the inner tube and out the common orifice of the two tubes, thereby creating a partial vacuum in the space between the adjacent walls of the inner and outer tubes. The small hole shown at the base of jet *A* leads to the reservoir of liquid which is drawn up into this space and is driven out into the flask with the air stream from the inner tube. The mixture of liquid and air is then met by the opposing air streams from jets *B* and *B'*. These two jets with orifices of approximately $\frac{1}{32}$ inch, are made of $\frac{1}{8}$ inch glass tubing and are joined together at the base. The placement of the two jets for producing best results was determined empirically. When the air jets are placed very close to the air-liquid jet the delivery of liquid is almost stopped; on the other hand, if they are fixed beyond the optimal distance much of the air-liquid mixture is by-passed around the opposing air streams and the additional effect of finer dispersion of the liquid particles is lost. A considerable portion of the atomized liquid

impinges on the surface of the Florence flask, the larger droplets returning to the reservoir while the small particles are carried out of the atomizer with the air current.

The operating efficiency of the atomizer is influenced by the dimensions of the air jets and the air-liquid jet and by the air pressure employed. Other factors, such as temperature and relative humidity of the air, the viscosity, boiling point, temperature, etc., of the liquid will also influence its performance. The occurrence of a distillation effect, inversely related to the humidity of the air introduced, has been noted. These problems are being investigated in our laboratories at this time. The following calibrations are tentative and have purposely

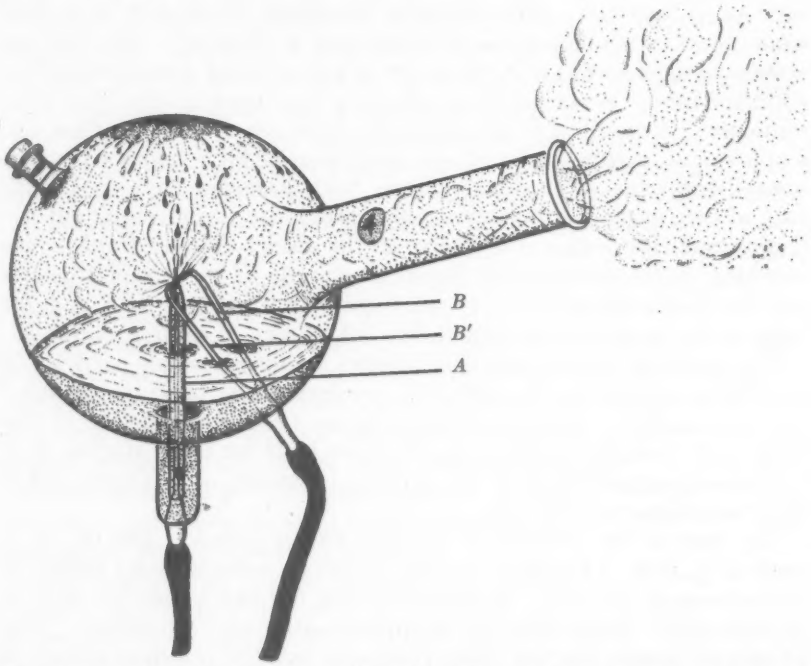

FIG. 1.—Atomizer which delivers uniformly small particles at rate of approximately 55 to 65 cc. per hour at pressure range readily obtainable in the laboratory.

ignored many of the complicating factors in order that rough performance data could be obtained and utilized for investigating direct immunization of the respiratory tree.

The curve shown in Figure 2 depicts the efficiency data for one of these atomizers at various air pressures. The calibrations were made at room temperature (21° C., relative humidity averaging 55%) using distilled water as the liquid. The air pressure is shown on the abscissa in terms of centimeters of mercury while the volume of water atomized per unit volume of air (cc./liter) is plotted on the ordinate. It will be seen that within the ranges of pressure from 20 to 55 cm. of mercury the calculated efficiency is expressed in a smooth curve. The efficiency

of the atomizer increases with each increment in operating pressure, but at a gradually diminishing rate. At 40 cm. of mercury the atomizer has an output of approximately 0.024 cc./liter of air delivered to the atmosphere. The upper pressure ranges were used most commonly because the greater output of the atomizer permits more rapid attainment of equilibrium in the experimental chamber.

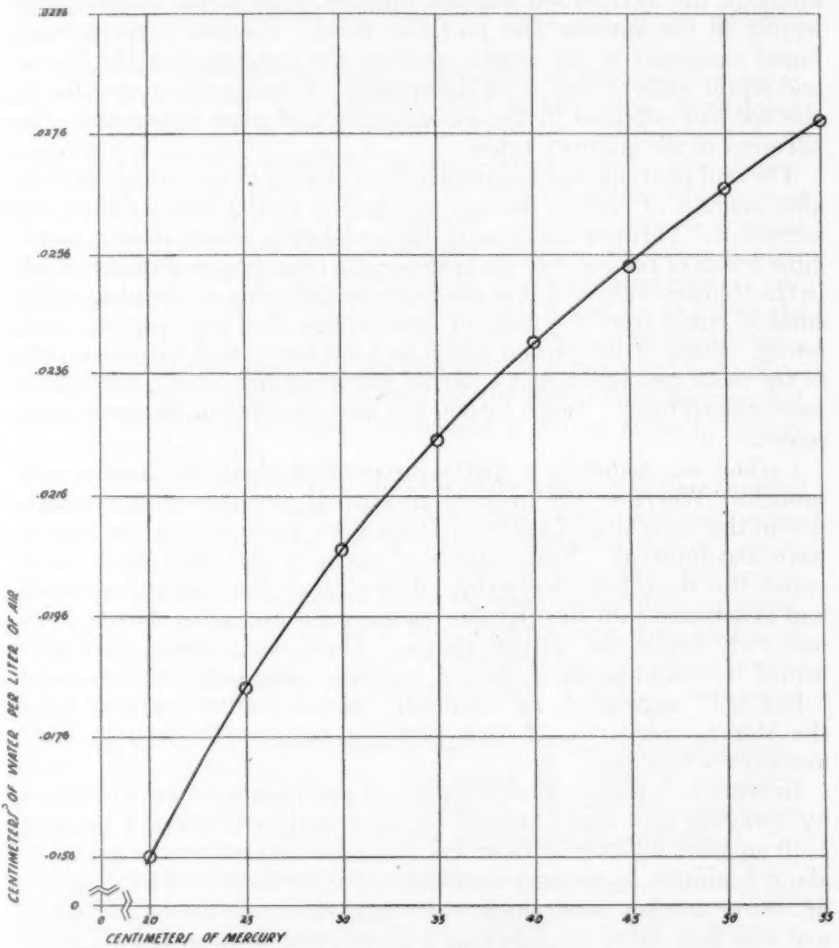

FIG. 2.—Efficiency curve—centimeters³ of water delivered per liters of air.

It has not been deemed necessary to determine accurately the particle size delivered by the atomizer at this time. Although several methods are described in the literature we have not found one which we feel would be sufficiently accurate to justify such determination.

In order to investigate the extent to which a suspension atomized through the apparatus described would penetrate into the lung substance, a full grown *Macacus rhesus* monkey was confined in an atmosphere of India ink sprayed in a 1:10 dilution for 1 hour. The air

pressure was held at 40 cm. of mercury. Upon completion of the atomization the animal was chloroformed and autopsied immediately to determine the carbon distribution.

No carbon was observed in the mouth, thus suggesting that most of the inspired vapor had been taken in through the nose. The vestibules of the nares and the nasal cavities in general contained carbon, although the distribution was not uniform, more of the particles collecting in the anterior and posterior thirds. Carbon particles were found entangled in the mucus covering the membrane of the lateral and septal walls as well as on the conchæ. Considerable quantities of the ink had collected in the nasopharynx and some appeared in the openings of the auditory tubes.

The oral pharynx had apparently been cleared of any grossly detectable amount of carbon through swallowing of the salivary and oral secretions. This was also true of the esophagus, which showed negligible traces of carbon but the presence of a considerable amount of ink in the stomach indicated that the particles collecting on the pharyngeal surface, either from the nasal or oral cavities, had been rapidly swallowed. Some of the carbon which had not been caught in the mucus of the nasal passages found a partial barrier in the vocal cords, which were stained black. Some carbon was also observed in the ventricular recess.

Carbon was found in a spotty distribution along the trachea and bronchi. Wherever the inspired air current had turned at an angle due to the branching of the respiratory tract, more carbon was seen to have accumulated. Examination of slices of the fixed lung tissue under the dissecting microscope showed that the inspired material had penetrated into the alveolar spaces and was readily detected immediately below the visceral pleura. These observations were confirmed by examination of stained sections. The latter also revealed "dust cell" aggregates, an essentially normal feature resulting from the chronic inhalation of dust particles and usually seen in most monkeys of this age.

In order to compare the distribution of particulate material obtained by spraying with that obtained by intranasal instillation, 4 cc. of a 1:10 aqueous dilution of India ink was administered over a period of about 5 minutes by dropper instillation into the nostrils of a full-grown *M. rhesus* monkey maintained under light ether anesthesia. The animal was then killed by injecting 2 cc. of ether intracardially and an autopsy performed. The lungs were removed in continuity with the bronchi, trachea and larynx, and the air passages from the epiglottis to the smallest bronchi were then laid open with the scissors. Considerable carbon was found entangled in mucus in the larynx, especially above the vocal cords. The visible distribution of carbon in the trachea was irregular, a few black patches of carbon-containing mucus being found at intervals along the tract. Occasional minute deposits of ink could be detected within the smaller bronchi, especially in the middle and lower lobes. There were in all not more than 10 areas of diffuse blackening in the respiratory tissue proper. How much carbon

had reached the alveoli was not ascertained, but it was quite evident that it was very small compared with the amount seen in the lungs of the animal which had been sprayed.

No carbon could be detected on the pharyngeal mucous membrane, nor in the upper half of the esophagus. The lower half of the esophagus contained an abundance of the carbon-mucus mixture and in the stomach there was estimated to be more than half of the administered ink.

A considerable amount of carbon embedded in mucus was found in the nasopharynx, and the posterior third of the nasal passageway was also congested with black mucus. This was likewise true of the vestibule and the anterior part of the nasal cavity proper. The mucous membrane covering the middle third of the turbinates, septum, and lateral walls was relatively free of carbon. Judging from the amount of carbon found to have been swallowed within a few minutes after introduction into the nares, the bulk of the carbon-mucus mixture still remaining in the nasal cavity would also have found its way into the alimentary tract in a short time. The carbon-free zone of oral pharynx and upper esophagus points to the operation of a rapid and very effective swallowing mechanism for clearing the particulate matter that becomes grounded in the nasal mucus and is moved along by ciliary action to the pharynx.

One inescapable conclusion is that the intranasal instillation method provides a rather direct route to the alimentary tract, especially when fluid is introduced that may act to incite an increased flow of mucus. The inhalation of a finely vaporized spray directly into the larynx through the mouth should permit much less swallowing of the material.

In order to determine roughly the proportionate distribution of inspired particulate matter, a third experiment was carried out using a suspension of radio-active chromic phosphate (Cr_2PO_4)* in place of the India ink.

The tidal air of a *M. rhesus monkey* (weight, 7¾ pounds) under intravenous nembutal anesthesia was determined by means of an intratracheal cannula connected to a respirometer. Several determinations were made and the consecutive results are presented in Table 1.

TABLE 1.—TIDAL AIR DETERMINATION (*M. rhesus*).

Time of measurement (min.)	Average respirations per min.	State of breathing	Air per minute (cc.)
18.0	32	Shallow	553
3.6	26	Deeper; regaining consciousness	905
(More nembutal administered)			
2.4	30	Deep; partially conscious	769
18.0	26	Shallow	543
13.6	25	Shallow	542

It is evident that if the animal is kept at a constant level of anesthesia its tidal air can be determined and close checks obtained. The

* Obtained through the courtesy of Dr. Hardin Jones, Donner Medical-Physics Laboratory, University of California, Berkeley, Calif

average for the three determinations (1, 4 and 5) is 546 cc. per minute. As soon as the animal regains partial consciousness the tidal air increases markedly. It was not possible to obtain figures for tidal air of the fully conscious monkey.

The next day the same monkey was put under intravenous nembutal anesthesia and placed in a chamber which had previously been subjected to a spray of radio-active chromic phosphate for 37 minutes to bring the concentration of the fog to equilibrium. The animal was exposed to the spray for 40 minutes. During the 77 minute total spraying 79 cc. of the material, containing 0.1 microcurie per cc., were atomized at a pressure of 40 cm. mercury. At the termination of this period the monkey was killed and autopsied.

Except for a region extending about 4 mm. within the nasal vestibule, where a yellowish-gray deposit was observed, no chromic phosphate could be detected grossly throughout the body of the animal. There were no signs of inflammation or hyperemia in the respiratory or alimentary organs.

A small section from the apical region of the lung was frozen and dried *in vacuo* and a free-hand razor blade section weighing 5 mg. made. This was laid out on a piece of blotting paper covered with cellophane and tested against a Roentgen ray film for beta ray emanations. After a 3 day exposure a picture was obtained, indicating that finely atomized particulate matter was uniformly distributed throughout the lungs, the image of the print following the exact outline of the tissue section tested.

The lungs, dissected free from adjoining tissue and separated from the trachea, weighed 24 gm. They were frozen and dried *in vacuo* and then incinerated in a flat porcelain dish at a temperature which left a residue of ash including the chromic phosphate. This ash was analyzed for radio-activity by means of a beta-counter, and the value obtained used to determine the total amount of atomized chromic phosphate remaining in the lungs after inhalation.

Beta counts made on the chromic phosphate residue remaining in the atomizer after atomization showed an increase of 100% from 0.1 to 0.2 microcurie per cc., indicating that a distillation effect was taking place during the spraying. By calculation of the amount of radio-activity contained in the 97 cc. of the original sample introduced into the atomizer and the amount remaining in the residue, it was possible to determine the average activity of the 79 cc. which were delivered to the atmosphere of the monkey. From the known figures on air and fluid delivery of the atomizer and the previously determined figures on tidal air under nembutal anesthesia it was calculated that the monkey had inspired a total of 0.59 cc. of radio-active material. By radio-activity measurements of the lungs of the same monkey it was calculated, by comparison with a control chromic phosphate sample, that approximately 0.1 cc. of the material or 0.01 microcurie had been retained in the lungs. In view of the fact that a second monkey treated similarly showed that of the total amount of radio-active material contained in the body, 75% was in the respiratory tract and only 25%

in the alimentary tract, the difference between the amount calculated to have been inspired, 0.59 cc. and the amount retained, 0.1 cc., must be considered to be due chiefly to the material exhaled. Heubner[14] states that between 50% and 80% of an inhaled suspension is exhaled by man. With an undeterminable variable of such magnitude at play it is difficult to assess the accuracy of our figures until repeated confirmations are obtained. Theoretically, using a radio-active substance as an indicator would seem to be a relatively accurate experimental method to determine such values for animals.

Another radio-autographic print was made with a teased lung section from one of a group of mice exposed to radio-active chromic phosphate for 1 hour, and showed the same penetration and uniform distribution of atomized particles in the lung tissue proper.

The data summarized above offer such clear-cut evidence of the superiority of the inhalatory method that it was considered advisable to employ it in experiments designed to test the value of influenzal antiserum for the protection and treatment of man.

Summary. 1. An atomizer has been described which is capable of producing a fine particle mist with high efficiency in terms of volume of atomized liquid per unit volume of air delivered. Preliminary performance data are presented.

2. India ink and radio-active chromic phosphate were used as indicators in experiments on monkeys and mice to compare the inhalatory method and the intranasal route. The superiority of the inhalatory method with respect to distribution and penetration of both materials was definitely established.

II. Immune Serum in Prophylaxis and Treatment*

By the Personnel of Naval Laboratory Research Unit No. 1

Introduction.—The application of immune serum directly to the respiratory tract for the purpose of conferring a passive immunity is not a recent innovation. Besredka[2] as long ago as 1920 advocated intratracheal injections of immune serums for development of a general passive immunity. He claimed that this method would not only confer a strong, immediate immunity, but would obviate the danger of anaphylactic shock in those cases which would be susceptible to the usual intravenous serum administration. However, he failed to compare the resultant immunity from intratracheal inoculation with that produced by equal amounts of serum administered intravenously, or intramuscularly. Jones[15,16] demonstrated that the intratracheal route does not compare favorably with intraperitoneal inoculations in ability to confer a general passive immunity.

Fox,[9] in a further investigation of the problem, confirmed and extended the observations of Jones that, although intratracheal administration of antiserum produces a definite blood antibody level, it is "a

* We wish to express our sincere thanks to the Cutter Laboratories and particularly to Mr. L. B. Blackledge of that organization for the very considerable help accorded us in fractionation of the anti-influenzal horse serum.

grossly inefficient way to induce a general passive immunity" when compared with the intravenous or subcutaneous routes. In the course of these experiments it was demonstrated that antibodies appear in the blood stream only slowly and in low titer, and, conversely, that these antibodies are retained in the lungs as long as 5 days. This definite indication of the relative impermeability of the lungs to antibody protein has been further corroborated by the report of Drinker, Warren and MacLanahan[7] whose work demonstrated that proteins such as horse serum and crystallized hemoglobin were absorbed but slowly from the lungs and, contrary to expectations, invariably appeared first in the blood rather than in the lymphatics.

Fox[9] has quite clearly stated the case for the respiratory route of administration of immune serums. He writes: "In pulmonary diseases amenable to serum therapy certain benefits accruing to local use of serum are suggested. In addition to the possible reduction of the peril of anaphylaxis as suggested by Besredka, one may enumerate particularly (1) the much higher local antibody content of the lung obtainable after local administration of small amounts of serum than follow injection of much larger amounts intravenously, and (2) the prolonged retention of such antibody."

To turn to our problem of influenza, we may concern ourselves with the arguments for and against the use of immune sera administered locally for the prevention and treatment of this disease. The most obvious advantage of such a method would be the immediate, albeit fleeting, production of an immune state, thus supplying a rapid means of combating an epidemic in a susceptible group. Were successful vaccination against influenza a *fait accompli*, enabling us to minimize significantly the susceptibility of our almost universally susceptible population, we might regard serum prophylaxis as unwarranted, but at the present time the status of influenza vaccines is not too encouraging. The reports of Russian workers[18,30,31] employing horse serum both prophylactically and therapeutically indicate the value of the treatment while little mention is made of contraindications or untoward reactions.

Theoretically, however, there would seem to be some danger involved in the repeated inhalation of a heterologous serum. It is highly probable that sensitization to the foreign proteins of the serum would result in man, if we can consider the reaction to be comparable to that of the guinea pig even in a qualitative manner. Sensitization and fatal shock by inhalation techniques[1,4,11,26,28,29,33] have been repeatedly observed in this animal. To minimize the risk of allergic response in man, the ideal serum for use as an inhalatory immunizing agent would be human convalescent or hyperimmune serum. However, it is difficult to obtain large quantities of this material in sufficiently high titers. Rabbit serum might be less likely to cause sensitivity reactions than horse serum, but here again for the quantities of serum needed in any large scale operations the rabbit is not as satisfactory as the horse. Nevertheless, this fact may be outweighed by the higher immune titers that can be produced in the rabbit.

The use of a salted-out globulin fraction of horse serum, although enabling a significant concentration of antibodies to be made, does not eliminate the sensitivity problem. Recently developed methods of purification and despeciation of immune sera by enzyme digestion would seem to have application here.[6,17,20,23,24,25,36] Such methods of purification alter the specificity of the serum proteins to such an extent that cross-sensitivity reactions to the original serum are greatly reduced. At the same time the digestion process brings about an actual reduction in antigenicity of the proteins involved, as demonstrated by large quantitative differences in sensitizing and reacting properties. The inevitable drop in antibody content of the despeciated serum can be tolerated providing subsequent concentration would raise the product to an effective potency.

In view of the facts that the absorption of proteins from the lung into the blood is a slow process and the bronchial tree of man is not heavily coated with smooth muscle as in the guinea pig, we should not expect to see acute shock resulting from inspiration of serum or serum fractions into the respiratory tree. There is, however, a definite possibility of delayed reactions of asthmatic nature appearing within a few hours time in hypersensitive individuals. Administration of material such as serum proteins by inhalation would be contraindicated in acknowledged asthmatics, while the number of reactions in others could very likely be greatly reduced by a thorough screening, *i. e.*, detection of serum-sensitive individuals by accurate history-taking combined with skin and conjunctival tests. Positive reactors conceivably may be desensitized by intradermal administration of serum or by the inhalation of successively larger amounts.

The danger of sensitization and subsequent production of reactions in a group of individuals subjected to treatment during an epidemic is decidedly reduced merely by the necessary timing of the inhalations. Since the passive immunity conferred by the serum is effective only for a relatively brief period it would probably require one, two, or possibly even three treatments per week to maintain an individual in the immune state. With such spacing of antigenic stimuli it is improbable that reactions would occur during a few weeks treatment, since each inhalation would serve to induce temporary desensitization and insufficient time would elapse between inhalations for the production of a true hypersensitive state. If it is practicable to employ a despeciated, purified product instead of whole serum or its globulin fraction, some of these speculative problems may be avoided. This aspect of the problem is being investigated at the present time.

In anticipation of clinical trials of immune serum or a fraction thereof to be administered by inhalation, certain laboratory experiments were undertaken to confirm and extend observations on the value of the method in prophylaxis and treatment.

Preparation of Horse Immune Plasma and Its Globulin Fraction. Horses were immunized by weekly subcutaneous inoculations of active mouse lung viruses. Sample bleedings were made at intervals and tested by a red blood cell agglutination-inhibition method[21] until a

sufficiently high titer was obtained, the first volume-bleeding being made 9 days after the tenth inoculation. The antigen inoculated consisted of 50 cc. of a mixture of PR–8, W.S., Philadelphia, and Lee strains of virus prepared in sterile broth in a concentration of 10% mouse lung by weight. Three bleedings were made from 1 horse, allowing time for one virus inoculation between the second and third bleedings, and 15 liters of plasma recovered. The presence of the fibrinogen in the plasma proved to be a troublesome factor, so that subsequently all bleedings have been performed to obtain serum.

The plasma was salted out by the sodium sulfate method and the fraction precipitating between 8% and 21% concentration was recovered and redissolved in a volume of 3 liters, or one-fifth of the original plasma volume. (In later fractionations 17.5% sodium sulfate was used as the upper limit, for the fraction between 17.5% and 20% apparently does not contain protective antibodies.) To this concentrate was added 0.3% tricresol as a preservative. This globulin fraction was light greenish in color and of a viscous consistency. Microkjeldahl determinations on the plasma and the globulin gave the following results for total nitrogen: whole plasma, 8.44 mg. nitrogen per cc.; concentrated globulin, 17.20 mg. nitrogen per cc.

Neutralization tests against 1000 MLD of PR–8 mouse lung virus gave the following comparative results, calculated according to the method of Reed and Muench:[27]

	50% mortality end-point	50% maximum score end-point
Whole plasma	1:865	1:250
Globulin	1:3450	1:1540

These results would indicate that most of the antibody content of the plasma had been recovered in the 8% to 21% globulin fraction, since the titer of the globulin, a five-fold concentrate, is approximately 4 times that of the original plasma by comparison of 50% mortality end-points and 6 times that of the plasma when 50% maximum score end-points are compared.

A neutralization test on the globulin run against 1000 MLD of F–99* egg virus gave a 50% maximum score end-point of 1:1380, although the viruses used to immunize the horse did not specifically include an F–99 strain. The globulin also exhibited strong protective powers against a Melbourne strain of virus, indicating the wide latitude of its antibody activity within the "A" type group of influenza viruses.

Protection of Mice by Intranasal Inoculation of Plasma or Inhalation of Globulin. It has been definitely shown that the administration of influenza immune serum by the intranasal route is more efficient than intra-abdominal or intravenous injection.[13,32,39] Zellat and Henle[39] further proved that mice could be protected against many lethal doses of virus if they first inhaled a serum spray for 2 hours.

It was our desire to confirm some of these results with respect to intranasal inoculation and inhalation. Accordingly, groups of mice

* Obtained through the courtesy of Dr. Joseph Stokes, Jr.

were sprayed for 30 minutes, 60 minutes, or 120 minutes with a two and one-half-fold dilution of our immune globulin. The 50% mortality titer of this diluted material can be considered as 1:1340. Each mouse was kept in an individual compartment within the spray chamber in order to prevent bunching of the animals during exposure to the globu-

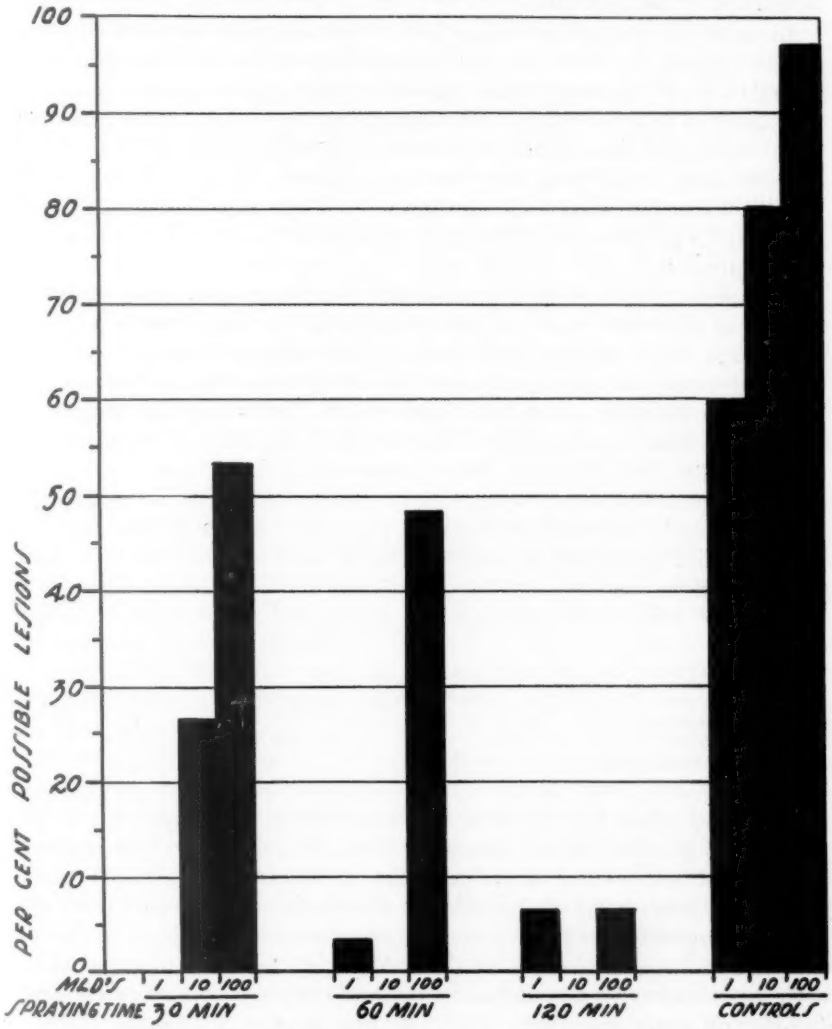

CHART 1.—Protection of mice by inhalation of immune globulin.

lin fog. Four hours after the spraying the mice were inoculated intranasally with graded doses of W.S. mouse lung virus. During a 10 day period of observation the animals succumbing were autopsied; at the end of this time all survivors were sacrificed and their lung lesions scored. Chart 1 is a graphic presentation of the results obtained. The vertical columns represent the per cent of possible lesions observed

in each group of 6 mice; for example, if the lesion score of a group of 6 mice adds up to 15, where death within 10 days is counted as 5 and the degree of lung consolidation in survivors is evaluated from 1 to 4, then the per cent of possible lesions observed would be 15/30 or 50%. It will be seen that the degree of protection increases with the time of inhalation of the globulin.

In order to compare the inhalatory route with intranasal inoculation another group of mice was inoculated intranasally at the same time with 0.05 cc. of dilutions of the concentrated globulin solution from 1:5 to 1:2560 in two-fold steps. By comparing the lesion scores resulting from intranasal inoculation of the same graded doses of W.S. virus 4 hours later it was computed that spraying for 30 minutes was comparable to an intranasal inoculation of 0.05 cc. of a 1:232 antiserum dilution, 60 minutes was equal to a dilution of 1:74, and 120 minutes to a dilution of 1:16.

The inhalation of the globulin did not compare as favorably with the intranasal inoculation as had been expected. It was suspected that a distillation effect taking place during atomization might account for the discrepancy and an experiment was set up to investigate this factor. A low titer immune serum was sprayed for 1 hour and the spray collected in a flask immersed in cellosolve and dry ice. Neutralization tests against 1000 MLD of PR–8 mouse lung virus were run on the original serum, the serum remaining in the atomizer after the spraying, and the serum collected during the spraying. Serum dilutions from 1:8 to 1:512 prepared in two-fold steps were used. Table 2 is a summary of the results.

TABLE 2.—SUMMARY OF RESULTS

	50% maximum score point	Total lesions per group	
		Ratio	Per cent
Original serum	1:69	97/210	46.2
Atomized serum	1:60	109/205	53.2
Residual serum	1-140	73/205	35.6

Measured either by the 50% maximum score end-point or by the per cent of possible lesions in each group, the residual serum showed the highest antibody content and the atomized serum the lowest. Here again is evidence of a significant distillation effect, a factor which must be considered in any estimations of inhaled material. The difference in lesions observed by Wells and Henle[37] between mice sprayed with virus and mice inoculated intranasally with the same calculated amount of virus may very likely be due to the distillation factor. The virus which reached their mice was probably less concentrated than their original material.

Another earlier experiment was designed to compare the duration and degree of protection conferred by a pseudo-globulin fraction of the horse plasma with that induced by whole plasma. The pseudo-globulin fraction was prepared by precipitation with 50% ammonium sulfate and dialysis against distilled water until sulfate-free. After removal of insoluble material by centrifugation, the supernatant was diluted

to the original volume of the plasma and brought to isotonicity by the addition of solid NaCl. Micro-kjeldahl measurements of the total nitrogen of these two preparations gave the following results: whole plasma, 8.44 mg. N/cc.; pseudo-globulin, 0.0837 mg. N/cc. Two groups of mice were inoculated respectively with 0.05 cc. aliquots of the plasma or its pseudo-globulin fraction administered by the intranasal route. At intervals of 24 hours, 72 hours, 6 days and 11 days following this treatment batches of mice from each group were tested for immunity by intranasal inoculation of graded doses of W.S. mouse lung virus.

The results are graphically presented in Chart 2, from which it is seen that the whole plasma was markedly superior to the pseudo-globulin fraction in protective power. By the 6th day following the inoculation of the plasma the passive immunity had all but disappeared, showing significant protection against only 1 MLD. These results are similar to those obtained by Henle, Stokes and Shaw[13] using immune mouse serum intranasally. They were able to demonstrate a slight immunity against 10 MLD as long as 10 days after the intranasal administration of the homologous immune serum.

Treatment of Infected Mice With Plasma or Globulin. Although the value of immune serum administered by way of the respiratory tract for the treatment of experimental influenza has been demonstrated by other workers, the question of whether repeated treatments are indicated has not been thoroughly investigated in the laboratory. Hare[12] conducted experiments in which two separate intraperitoneal injections of immune ferret serum were given 24 hours apart to previously infected mice, but direct comparisons with other numbers of treatments were not made.

In order to determine more accurately the value of repeated instillations of immune horse serum the following experiment was conducted.

Mice were inoculated intranasally with 10, 100, or 1000 MLD of PR–8 mouse lung virus. At three different time intervals after infection, 6 hours, 30 hours and 72 hours, serum treatment was begun. Each treatment consisted of an intranasal inoculation of 0.05 cc. of the immune serum. The groups of mice were further divided into those receiving 1, 2, 3, or 4 serum treatments, 24 hours elapsing between successive serum instillations. Control mice were inoculated with normal horse serum, 4 instillations for each control group.

The results of this experiment are shown in Chart 3. Each vertical bar represents 10 mice, except in a few instances where non-specific deaths reduced this number to 8 or 9. The importance of repeated administrations of serum in the treatment of influenzal infection is significantly shown by this experiment. It is especially evident in the groups "30 hours, 10 MLD's," "6 hours, 100 MLD's," and "6 hours, 1000 MLD's." In the first instance there was a reduction of lesions from 74% (1 treatment) to 30% (4 treatments); in the second example, a reduction from 22% to 4%, and in the third, from 49% to 8% of possible lesions. In the groups of mice where the serum treatment was not initiated until 72 hours after the virus inoculation, the infec-

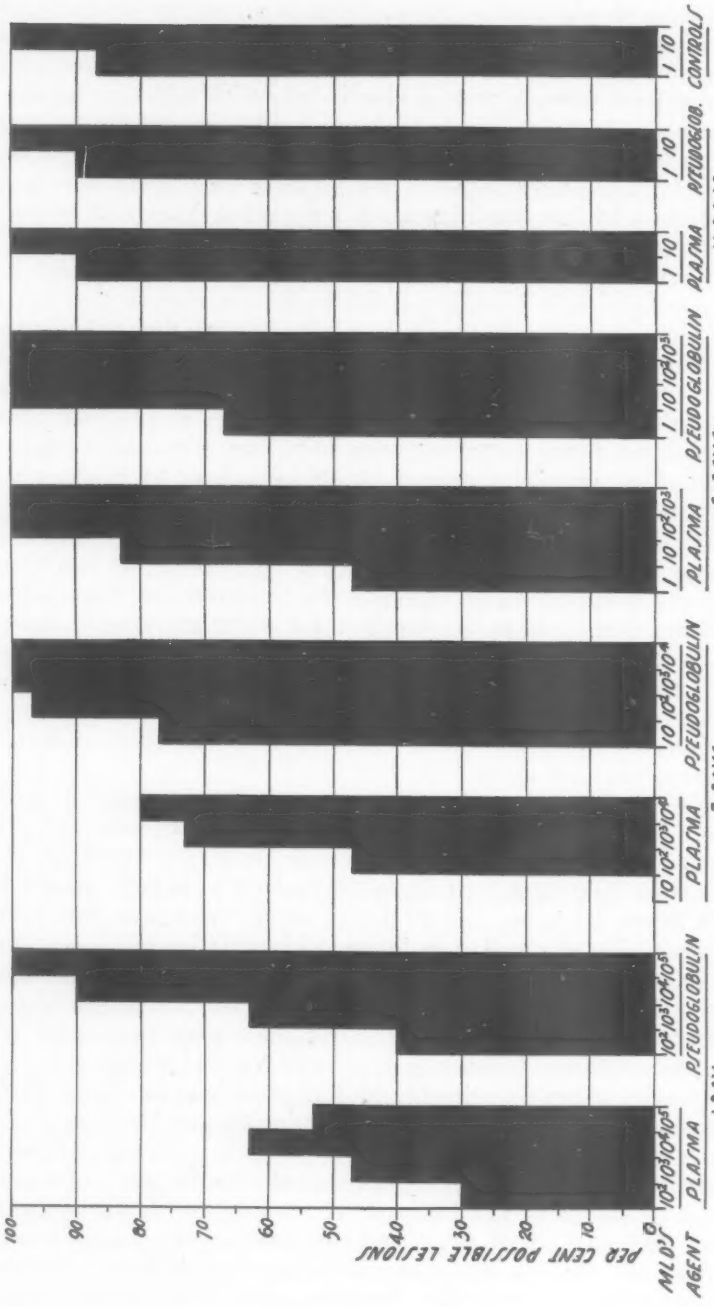

CHART 2.—Protection of mice by intranasal inoculation of immune plasma or pseudoglobulin.

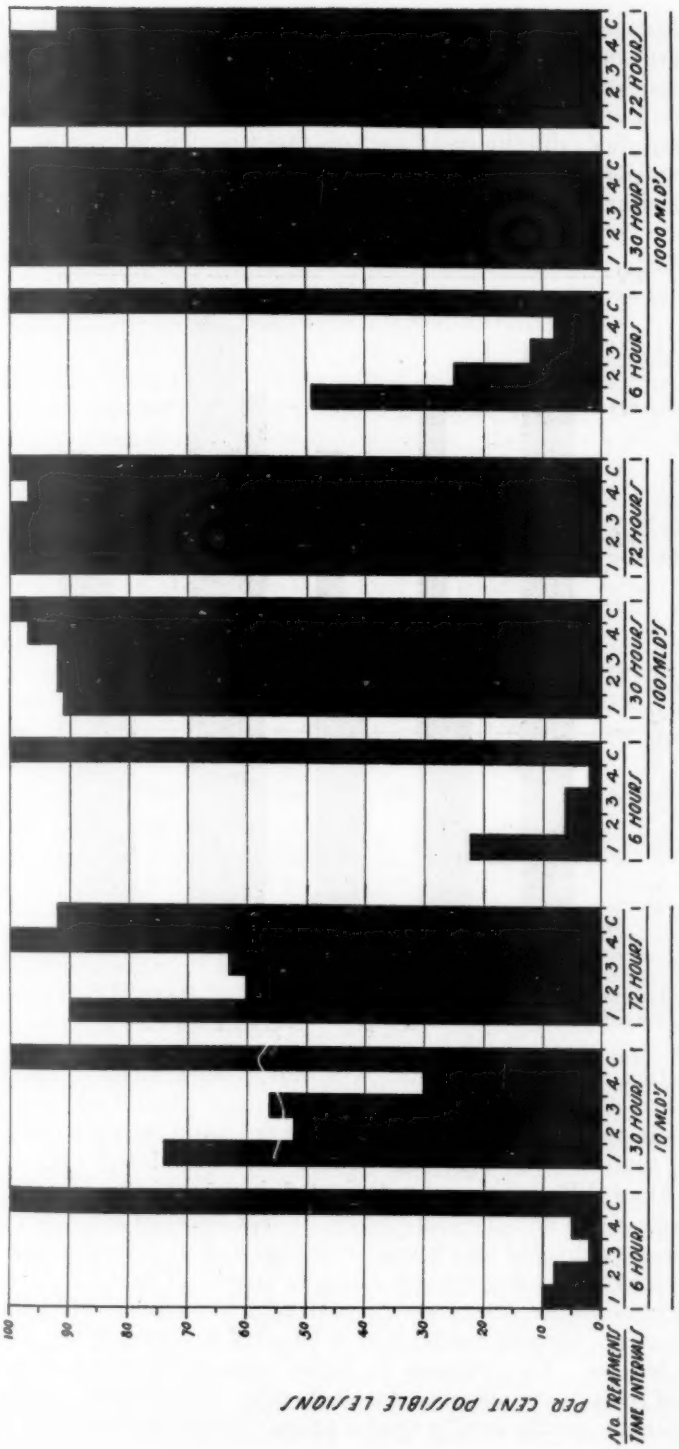

CHART 3.—Treatment of mice by intranasal inoculation of immune serum.

tion had progressed so far by the third or fourth serum instillation that the etherization and intranasal inoculation were not well tolerated. This is evidenced, for example, in the group receiving 10 MLD followed by 4 serum treatments starting at 72 hours. All the mice in this group died, due in great part to the repeated inoculations and administrations of anesthetic. The value of the successive serum treatments is clear, however, from the groups of mice in which treatment was begun earlier.

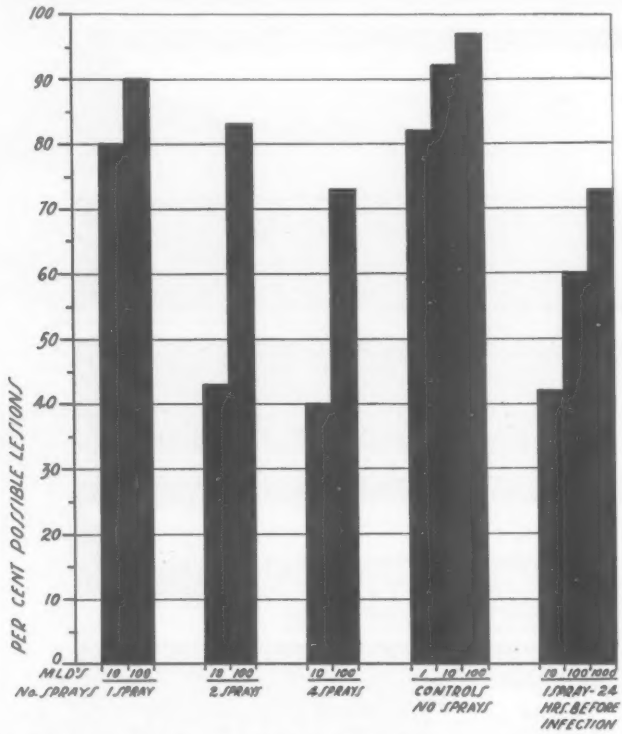

CHART 4.—Treatment of mice by inhalation of immune globulin.

Inhalation of immune globulin was also demonstrated to result in reduction of the lesion score in a group of mice which had been previously infected. Successive treatments were again shown to be more effective than a single spraying. Mice were inoculated with 10 or 100 MLD of PR–8 mouse lung virus 6 hours before spraying for 1 hour with a two and one-half-fold dilution of immune horse globulin. The mice were divided into groups receiving 1, 2 or 4 treatments, the multiple sprayings being spaced 24 hours apart. Ten days after the infecting dose all survivors were autopsied and lesions scored in the usual manner. Results are plotted in Chart 4 in terms of per cent of possible lesions. The decrease in lesion score with increase in the number of treatments, although not impressive, is consistent. Included for comparison are the results from a group of mice sprayed for 1 hour

with the same globulin solution and infected intranasally 24 hours later.

Attempt at Immunization With a Neutral Mixture of Serum and Active Virus. The consistent results obtained with serum in the prevention of experimental influenza by other workers as well as by ourselves led us to the possibility of using a serum-active virus combination in the hope that an active immunity would follow the short-lived passive immunity.

Preliminary experiments were performed in which mixtures of immune horse serum and allantoic fluid virus, non-infectious to mice upon intranasal inoculation, were instilled intranasally every 60 hours until 4 inoculations were made. Passive immunity was demonstrated 24 hours later and 2 weeks thereafter there seemed to be evidence of an active immunity having developed. In an attempt to confirm the results suggested in the preliminary work, the following experiment was performed.

Neutral mixtures of immune serum and PR–8 allantoic fluid virus, immune serum and Lee virus, and immune serum plus PR–8 and Lee viruses were incubated for 15 minutes at 37° C. The non-infectiousness of these mixtures was confirmed by mouse inoculation. A suspension of the serum plus normal allantoic fluid treated in the same manner was included as a control. Mice were inoculated with 0.05 cc. intranasally with one of the above mixtures every 48 hours until 4 inoculations were completed. Fourteen days later each group was tested for active immunity against both PR–8 and Lee mouse lung viruses by intranasal inoculation of from 10 to 10,000 MLD. Results were entirely negative and there was no evidence of an active immunity having developed.

The inability to produce an active immunity by combining serum with the virus is doubtless based upon the same mechanism noted in similar trials of combined vaccines against diphtheria and tetanus.[19,22] Although results are dependent to some extent on quantitative relationships and the time relationships between inoculations, the presence of a passive immunity, in general, tends to inhibit the normal development of an active immunity.

A later experiment designed to produce immunity in mice by intranasal inoculation of a formolized vaccine gave further confirmation of these results. Mice were divided into 2 test groups, the first group receiving 0.05 cc. of formolized PR–8 allantoic fluid virus intranasally every other day until 5 inoculations were completed, the second group receiving the same course of formolized virus inoculations and also being inoculated with 0.05 cc. of immune horse serum intranasally on the alternate days. This second group of mice thus received 5 formolized virus inoculations and 4 serum inoculations. Ten days after the last inoculation both treated groups, as well as control mice of the same age, were tested for active immunity by intranasal inoculation of PR–8 mouse lung virus. The results are presented in Chart 5 and demonstrate the definite establishment of immunity in mice inoculated intranasally with inactive virus, but a lack of immunity in the mice

which alternately received serum and vaccine. Although it has been demonstrated by Eaton and Beck[8] that with active mouse-adapted strains of virus inoculated intranasally it is necessary to produce rather severe lung lesions in order to induce an immune state, an inactive virus

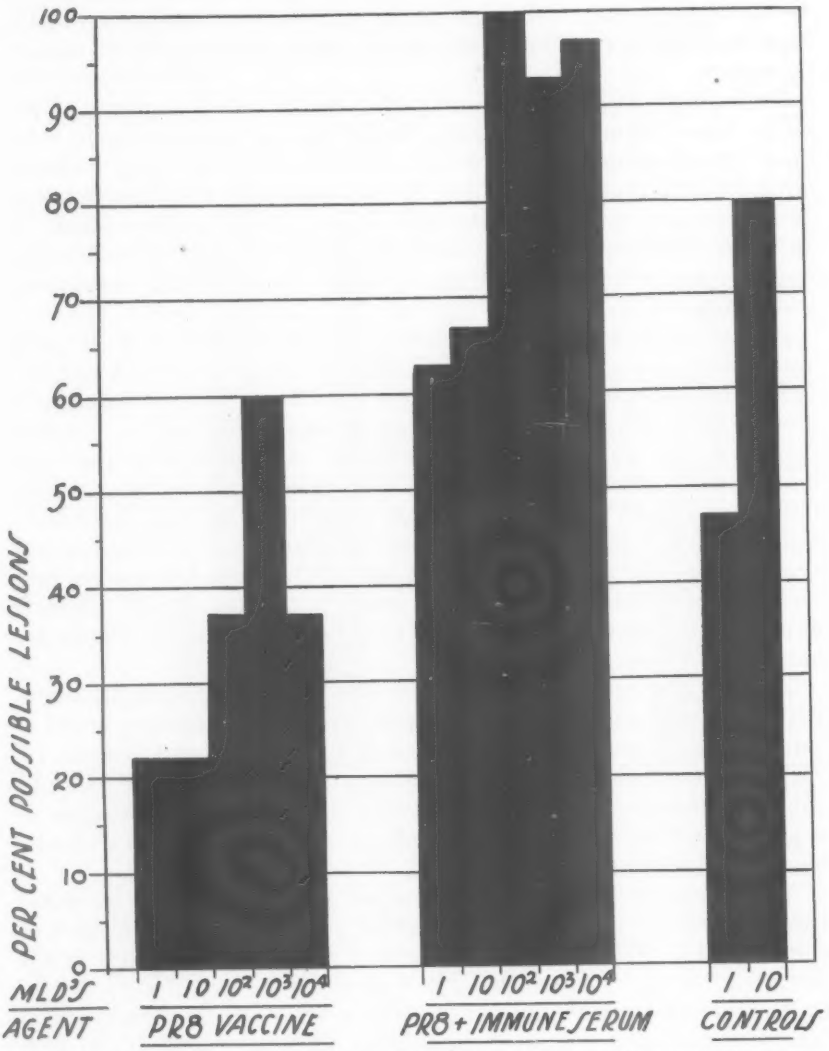

CHART 5.—Immunization of mice by intranasal inoculation of formolized virus alone or in combination with immune serum.

is capable of immunizing mice intranasally with no injurious effects. The inhalation of inactive virus by mice would very likely result in even better immunization because of the more uniform distribution of the antigenic material. This aspect of the problem is now under investigation.

The reports of Walsh and Cannon[5,34,35] are of interest in this regard, for they indicate that the ratio of tissue antibodies to serum antibodies is consistently higher following intranasal vaccination than that resulting from a general immunization. Their antigens were bacterial in nature, but it might be anticipated that the same principle would apply in the case of influenza virus. Their belief is that local formation of antibodies is responsible for the higher concentrations found in organs of the regionally immunized animals and that regional vaccination would seem therefore the preferable method for elevating the level of resistance of tissues at a portal of entry to the body. Therefore, the direct administration of an influenza vaccine to the respiratory tract should be carefully considered as a possible method of choice. The question of the possible use of attenuated virus to produce a subclinical infection as a method of immunization has been discussed recently by Francis[10] and Burnet and Clark.[3]

Summary. 1. High titer horse immune serum or its globulin fraction, administered either by intranasal inoculation or by inhalation, protected mice against subsequent intranasal infection with influenza virus. The degree of protection conferred increased with the time of exposure to the globulin spray.

2. Atomization of solutions through nebulizers of the type employed results in a distillation effect, which must be taken into consideration in quantitative estimates of inhaled materials.

3. Whole immune plasma was superior to any individual globulin fraction in the degree and duration of its protective power for mice.

4. Treatment of mice with horse immune serum intranasally, or globulin by inhalation, is effective in reducing the lung lesions. The necessity for early treatment is confirmed. The value of repeated treatments in lessening the severity of the experimental disease is definitely established, the lung lesions decreasing as the number of treatments is increased.

5. Neutral mixtures of immune serum and active virus were ineffective in producing an active immunity in mice.

6. Mice subjected to repeated intranasal inoculations of a formolized virus showed a considerable degree of immunity when tested 10 days following the last inoculation. Immunity failed to develop in mice receiving concurrent intranasal serum treatments along with the formolized virus.

7. The prospective application of passive immunization of humans for the prophylaxis and treatment of humans is discussed.

ADDENDUM.—Considerable newspaper and magazine publicity containing inaccurate and unwarranted claims relative to our findings has preceded publication of this article. The authors were in no way responsible for this and take this means of expressing their disapproval of the resultant publicity.

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YEAST-LIKE FUNGI IN THE INTESTINAL TRACT OF CHRONICALLY INSTITUTIONALIZED PATIENTS

By Oscar Felsenfeld, M.D., M.Sc.
SUPERVISING BACTERIOLOGIST, DEPARTMENT OF PUBLIC WELFARE OF ILLINOIS
CHICAGO, ILLINOIS

(From the Research and Bacteriological Laboratory of the Chicago State Hospital)

The paramount problem confronting the bacteriologist in institutions with notably large bed capacity, such as state hospitals or penitentiaries, is the control of enteric infections.

It is a matter of regret that both for the therapy and prevention of such enteric disturbances, the finding of bacteria or parasites commonly considered as pathogenic does not provide at all times a secure guide. The negative findings of the usual clinical and microbiologic examinations in many cases of diarrhea prompted us to check not only the "pathogenicity" of intestinal microörganisms, but also to extend

our efforts to the search for possible new agents. Investigating the question of mycotic infections, it was found that little work had been conducted in the field of enteric mycology within the past few years. We therefore considered it our first task to investigate the "normal" flora of the intestinal tract in a large number of institutional patients.

Our main interest was concentrated on the Monilia-Cryptococcus-Geotrichum group. The Penicillum and Aspergillus species were neglected because contamination of the specimens could not be fully precluded. The simple and efficient method of Schnoor[3] as well as the nomenclature adopted by him were used. Thus we followed the classification of Moniliæ according to Martin *et al.*[2] Several attempts were made to differentiate Cryptococcus strains according to Benham and Hopkins[1] into 4 groups. The morphology of the colony, however, did not seem to be a sufficient guide. We found that due to the lack of serologic means a classification of these organisms was not possible. Torula, Saccharomyces and similar organisms were only occasionally encountered, and are not reported in this paper. Only 11 such strains scattered throughout the examined groups were cultivated.

Three groups of hospital patients were selected for the investigation, none of whom were afflicted with a skin disease. The stool specimens were collected from patients of the Manteno and Chicago State Hospitals.

1. The first group of cases included the stool specimens from 300 newly admitted patients and 600 cases from chronic wards, all of them without intestinal disturbances. This material was chosen in order to determine whether the intestinal flora changed during the hospitalization.

2. The second group consisted of specimens from 100 chronic typhoid carriers.

3. The third group was composed of diarrhea cases. (*a*) Stool specimens from 47 patients with *Shigella sonnei* and *Shigella paradysenteriæ* infections in the early acute stage. (*b*) Stool specimens from 51 proctoscopically confirmed* cases of bacillary dysentery 1 week after the termination of the sulfathiazole treatment. (*c*) Finally, the stool specimens from 103 cases of "food upset" occurring in 3 groups. This latter series consisted of patients who had shown a short period of diarrhea (from 4 to 8 hours) with only a few bowel movements, revealing light-colored stool without blood, mucous or other pathologic constituents. Bacteriologic, parasitologic and proctoscopic examination failed to disclose the cause of the upsets.

The results of the examination may be found in Table 1.

In comparison with the findings of Schwartz and Jankelson[4] and Schnoor[3] a higher percentage of Monilia and a lower percentage of Geotrichum were found in patients newly admitted to the hospitals. There were only a few patients who harbored 2 species of the group. In cases hospitalized for a longer period of time the incidence of Monilia, Cryptococcus and Geotrichum increased in the following man-

* The proctoscopic examinations were performed by Dr. L. H. Block, Consultant Proctologist of the Illinois Department of Public Welfare, Hospital Service.

ner: The number of cases excreting these organisms did not increase essentially, but many cases already harboring 1 member of the group became infected with a second or eventually also with a third species. In the absence of proper serologic tests, it is difficult to decide what immunologic process determines one person to be a ready host and a second person a less favorable host to these organisms.

TABLE 1.—ANALYSIS OF FUNGI FOUND IN DIFFERENT TYPES OF PATIENTS

Fungi found	Types of cases examined											
	New admissions		Ward cases		Typhoid carriers		Food upset		Bacillary dysentery		Treated dysentery	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
No. of cases:	300		600		100		103		47		51	
Monilia												
albicans	58	19.3	226	37.7	34	34	42	40.8	21	44.6	6	12
parapsilesis	13	4.3	26	4.3	3	3	1	0.9	2	4.2	1	2
krusei	7	2.3	16	2.7	4	4	1	1.9	1	2.1	0	0
others	1	0.3	3	0.5	2	2	2	1.9	2	4.2	0	0
Monilia	79	26.3	271	45.1	43	43	47	45.6	26	55.3	7	14
Cryptococcus	72	24.0	235	39.2	35	35	36	34.9	15	34.9	6	12
Geotrichum	69	23.0	177	29.1	26	26	58	56.3	25	53.1	3	6
Distribution												
Negative	80	26.6	146	24.6	26	26	15	14.6	8	17.0	37	74
1 species present	206	68.6	272	45.3	48	48	38	36.8	17	36.1	10	20
2 species present	14	4.7	155	25.8	22	22	47	45.6	17	36.1	3	6
3 species present	0	0	27	4.5	4	4	3	3.9	5	10.6	0	0

Typhoid carriers did not present a noticeable difference from the "chronic ward" patients.

In the "food upset" cases the incidence of Geotrichum increased doubly. The increased number of patients infected with Geotrichum reduced the number of cases without infection. Nearly 50% of the patients harbored 2 organisms. There was no change in the incidence of Monilia and Cryptococcus.

Although the low number of cases with bacillary dysentery does not permit definite conclusions, it must be conceded that the statistics differ from "food upsets" by the increase of Monilia findings and by the rise of the number of patients harboring 3 organisms.

In cases of dysentery treated with sulfathiazole, the absolute and relative incidence of Monilia, Cryptococcus and Geotrichum decreased substantially. Statistically, Geotrichum disappeared in seven-eighths of the cases, Monilia in approximately two-thirds, and Cryptococcus in about five-eighths—an observation which may possibly prove of some value in the treatment of mycotic diseases.

Summary. Monilia, Cryptococcus and Geotrichum were studied in stool specimens of 300 new admissions, 600 chronic ward patients, 100 typhoid carriers, 103 cases of "food upset," 47 acute bacillary dysenteries before treatment, and in 51 cases of bacillary dysentery after 10 days of sulfathiazole treatment. During the hospitalization the number of patients harboring the aforementioned organisms did not

increase but many cases previously infested only with 1 organism developed into carriers of 2 organisms. Typhoid carriers did not show a differing picture. In "food upset" the number of cases with Geotrichum increased remarkably. In bacillary dysentery the number of patients with Monilia also rose. Sulfa treatment reduced the number of mycotic carriers, and especially those with Geotrichum, by a pronounced margin.

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THE NATURALLY OCCURRING BLOOD SULFOCYANATES AND THEIR RELATION TO BLOOD PRESSURE

By Abraham Trasoff, M.D., F.A.C.P.
CHIEF OF MEDICAL SERVICE NO. 2
AND
Norman G. Schneeberg, M.D.
RESIDENT IN MEDICINE
PHILADELPHIA, PA.

(From the Medical Wards and Laboratories of the Mount Sinai Hospital)

In 1925 Nichols[4] discussed the pharmacologic properties of the sulfocyanates. He regarded the salivary glands as their chief site of formation, from which they were secreted into the saliva, swallowed and absorbed into the "system" appearing in tears and gastric juice. The salivary content of sulfocyanates averaged 0.01%. He assumed that if 1 liter of saliva was formed in 24 hours the amount of sulfocyanates are found in blood and body fluids in fairly equal concentration varying from 0.03 to 0.06 mg. per 100 cc. and excreted in the urine and feces in the ratio of 1:2. Cerebrospinal fluid concentrations are either the same or lower than in the blood. Caviness and his associates[2,3] have recently reinvestigated the naturally occurring sulfocyanates in blood using a technique modified from Ravin[5] which revealed concentrations definitely higher (0.31 to 2.55 mg. KCNS per 100 cc. blood) than had been previously reported. Repetition of determinations in the same subject exhibited a marked constancy of sulfocyanate concentrations. In 241 individuals studied, the blood sulfocyanate concentration varied inversely with the height of the blood pressure. They therefore ventured in hypothesis that naturally occurring blood sulfocyanates function as depressor substances which serve to counterbalance the action of various naturally occurring pressor substances, thus representing one of the many factors concerned in the maintenance of normal blood pressure. The importance of these observations, which would necessarily modify our concept of the pathogenesis of hypertension, stimulated us to undertake the work to be reported.

Methods. We studied 95 hospital in-patients and, as subjects of the tobacco experiment several volunteer residents, internes, and nurses.

Blood pressure recordings (a mercury column or anaëroid sphygmomanometer was used in measuring the height of the blood pressure) represent the average of 3 or more readings on different days with the subject at complete bed rest for 10 minutes or longer. Subjects with very obese or very thin arms, those whose blood pressures varied appreciably on successive readings, and patients in cardiac decompensation, in shock, or the victims of myocardial infarction, or patients suffering with febrile diseases were excluded from the study because of obvious sources of error. The diastolic levels recorded are those of Phase IV.[1]

Caviness' criteria for grouping subjects according to blood pressure levels were followed with a single modification:

Below 106 systolic: hypotension;
106 to 130 systolic: normotension;
131 to 140 systolic: borderline;
141 to 200 systolic: hypertension;
200 and above systolic: severe hypertension.

Caviness[2,3] stressed the necessity of control of physical activity, rest and diet in order to obtain accurate and comparable results in different patients. He collected blood samples before breakfast and before any tobacco was used that day. We adhered to these criteria, but, in addition, found that it was not sufficient to interdict the use of tobacco to the period immediately preceding the drawing of blood because habitual tobacco smokers show definitely higher blood sulfocyanate levels than non-smokers. Therefore our blood samples were all collected at least 48 hours or more after the use of tobacco.

Technique. To approximately 10 cc. of blood serum an equal amount of 10% trichloracetic acid was added, thoroughly admixed, and allowed to stand for 20 minutes. To 5 cc. of the protein-free filtrate obtained by filtering this mixture, 1 cc. of 5% ferric nitrate solution was added and the sulfocyanate concentration in milligrams per 100 cc. of blood was read directly in an Evelyn photoelectric colorimeter using a filter wave length 440 u.[2,3] We also checked the accuracy of our results by recovery tests upon several occasions during the period of this work.

Results. In 34 subjects with normal blood pressure (average pressure 116/71) the average blood sulfocyanate concentration was 1.31 mg. KCNS per 100 cc. of blood with a range of 0.84 to 2.4 mg. In 20 hypotensive subjects (average B.P. 93/58) the average blood sulfocyanate concentration was 1.18 mg. (range 0.90 to 1.54). In the hypertensive group (average B.P. 178/98) 28 subjects showed an average concentration of 1.19 mg. (range 0.74 to 2.48). Ten patients were included in the severely hypertensive group (average B.P. 227/120); they exhibited an average sulfocyanate concentration of 1.12 mg. (range 0.76 to 1.50); only 3 patients were investigated in the borderline blood pressure group (average B.P. 137/88) and presented concentrations of 1.21, 1.65 and 0.96 mg. (average of 1.24). A graphic comparison of these findings with those of Caviness *et al.* is demonstrated in Figure 1.

The effect of tobacco smoking on the blood sulfocyanate concentration was determined. Eight normotensive non-smokers served as a control group with an average sulfocyanate level of 1.03 mg. (range 0.84 to 1.21). The blood sulfocyanate level in 8 habitual tobacco smokers with normal blood pressures was 1.91 (range 1.24 to 2.48 mg.). One of these subjects smoked an average of 4 cigarettes per day and her sulfocyanate concentration was 1.24, the lowest recorded for the

tobacco habitués. Another subject smoked 8 cigarettes daily and showed a concentration of 1.45 mg. The remaining 6 subjects smoked 20 or more cigarettes per day. Blood samples on the 8 tobacco smokers were obtained after a 24 hour period of abstinence from smoking.

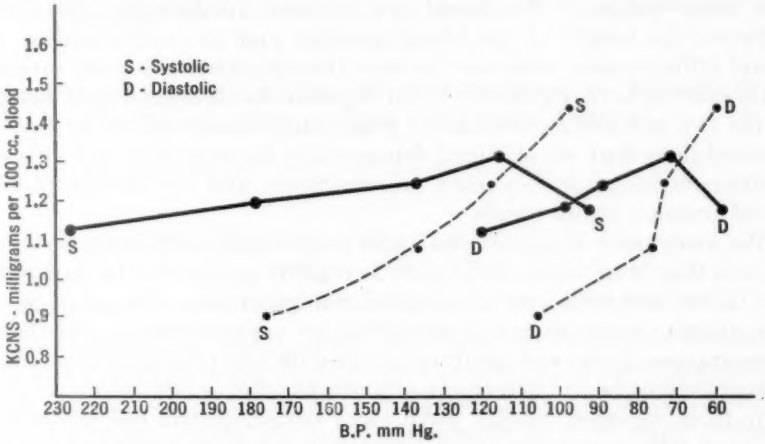

FIG. 1.—Blood pressure levels and blood sulfocyanate concentrations. Dotted lines represent the results of Caviness *et al.*; solid lines represent our results.

Excessive smoking for a 24 hour period produced no significant change in the sulfocyanate concentrations of these 3 subjects.

TABLE 1.—LACK OF EFFECT OF HEAVY SMOKING

	After 24 hr. period of tobacco abstinence	After 24 hr. period of heavy smoking (30–40 cigs.)
Subject A	1.75	1.80
Subject B	2.55	2.25
Subject C	1.90	1.91

The effect of a high-protein intake after a varying period on a low-protein diet was investigated in 2 cardiac patients. After an initial blood sulfocyanate concentration was obtained, these 2 patients were given a 100 gm. protein diet and 200 cc. of 15% amino acids intravenously daily. The results are tabulated below:

TABLE 2.—EFFECT OF CHANGES IN AMOUNT OF PROTEIN IN DIET

	Initial KCNS	48 hrs. on high-protein intake	7 days
Subject D . . .	1.21	1.07	0.98
Subject E . . .	1.44	1.29	1.24

Repetition of the sulfocyanate determination was done in 16 patients on different days. The greatest difference in any two determinations on the same patient was 0.37 mg.; the smallest difference was 0.01 (average difference for the group 0.16). In 5 subjects, 3 or more repetitions were performed which revealed a similar constancy of sulfocyanate concentrations in successive determinations.

Discussion. The blood concentration of the sulfocyanates obtained by us in normotensive subjects are almost identical with the values

reported by Caviness and his associates. They found an average concentration of 1.21 mg. KCNS per 100 cc. of blood (range 0.31 to 2.55); our average value was 1.31 mg. (range 0.84 to 2.40 mg.). We also noted a marked constancy of values in successive determinations in the same subject. We found no constant relationship, however, between the height of the blood pressure and the concentration of blood sulfocyanates, whereas Caviness demonstrated a definite inverse ratio existing between the two. In Figure 1 the discrepancy of results in the two series of experiments is graphically illustrated. The almost vertical lines that we obtained demonstrate the complete lack of correlation of blood sulfocyanate concentration and the height of the blood pressure in our hands.

We attempted to control our experiments identically with the conditions that obtained in their work as regards environmental and dietary factors and technique of chemical manipulations. Though most of our subjects were patients hospitalized for various reasons and theirs were apparently normal institutional inmates and prisoners, we cannot believe this to be the determining factor to explain our failure to confirm their reported results. We have demonstrated the uniformly elevated sulfocyanate blood levels in habitual tobacco smokers as compared to a control group of non-tobacco users. Caviness and his coworkers stated that their only precaution as regards tobacco smoking was to draw blood samples in the morning "before tobacco was used." Since we found that blood sulfocyanate levels remain elevated in tobacco smokers after a 24 hour period of abstinence this precaution is not sufficient to eliminate the effect of tobacco in habitual smokers. This may, in part, explain the elevated sulfocyanate blood concentrations in some of their subjects.

In the light of our findings we cannot regard the blood sulfocyanates as naturally occurring depressor substances nor can we regard sulfocyanate therapy in hypertension as a form of substitution therapy for a substance that, because of a deficiency in the blood, has allowed various pressor substances to act uninhibited resulting in hypertension.

Summary. 1. Sulfocyanates occurred normally in human blood in concentrations averaging 1.31 mg. per 100 cc.

2. Habitual tobacco smokers showed blood sulfocyanate concentrations well above the normal average.

3. No constant relationship of blood pressure to blood sulfocyanate concentration was found.

4. The naturally occurring blood sulfocyanates probably do not represent an important factor in the maintenance of normal blood pressure.

We wish to thank Dr. David R. Meranze, Director of the Laboratories, for the use of the Laboratory Facilities, and Joseph Schaffer, B.A. (Chem.) and Herbert Marks, B.A. (Chem.) for performing the sulfocyanate determinations.

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TREATMENT OF 134 CASES OF MENINGOCOCCIC INFECTION WITH MASSIVE DOSES OF SULFADIAZINE

By Major Bruno A. Marangoni, M.C., A.U.S.
and
Major Vincent C. D'Agati, M.C., A.U.S.
Fort Mc Clellan, Ala.

(From the Contagious Disease Section, Medical Service, Station Hospital)

The treatment of meningococcic infections has undergone considerable and important changes since the introduction of the sulfonamide drugs. Palliative treatment was the keynote of therapy after the isolation of the meningococcus by Weichselbaum in 1887 until Flexner introduced serum therapy in 1908. The use of antimeningococcus serum intravenously, intramuscularly, and often intrathecally, in conjunction with supportive and symptomatic measures, held unchallenged sway until 1936, when the sulfa drugs—sulfanilamide followed by sulfapyridine, and sulfadiazine—were demonstrated to be efficacious additions to the therapeutic armamentarium of meningococcic infections. Nevertheless, there is no uniformity of opinion as to the relative merits of serum and the sulfonamides; some investigators recommending the use of sulfonamides alone, others, a combination of chemo- and serotherapy.

During the pre-serum stage in the treatment of cerebrospinal fever, the mortality rate varied from 20 to 90%. Following the use of serum, mortality figures declined, ranging between 20 to 40% in most series reported.[9] During the first World War 5839 cases of meningococcic meningitis were reported by the Office of The Surgeon General with 2279 fatalities, a mortality incidence of 39%.[11] The discovery of sulfanilamide in 1936, sulfapyridine in 1938 and sulfadiazine in 1940 ushered in the third or chemotherapeutic phase in the treatment of meningococcic diseases. Dingle and Finland[3] presented an extensive review of the literature of meningococcic meningitis with comparative mortality rates for different combinations of therapy. In over 1000 cases treated with sulfanilamide alone, the mortality rate was 14%; in 300 patients treated with combinations of sulfanilamide and antiserum, 25%; in approximately 700 patients treated with sulfapyridine alone, 8%; and for 200 patients treated with sulfapyridine and serum, 12%. The number of patients treated with sulfadiazine was insufficient to permit appraisal and comparison with other sulfonamide drugs. The reason for the higher mortality rates when sero- and chemotherapy were used in combination seemed difficult to explain and evaluate. One plausible explanation is that this group of patients was more

seriously ill or had failed to respond to the sulfonamide drugs alone. On the other hand, mortality figures in meningogoccic infections have been consistently lower when treated by a combination of antiserum and one of the sulfa group than by the use of antiserum alone. Campbell[2] reported a series of 10 cases treated with serum and sulfanilamide with no deaths. This series is too small in itself to be of any statistical significance. Banks'[1] series of 65 cases were treated with sulfanilamide, terminating in 8 deaths, (12.3% mortality). In a recent editorial on the therapy of cerebrospinal fever in England,[4] the following statistics in all age groups were reported: treatment with serum alone gave a fatality of 36.6%; with sulfonamide compounds plus serum, 13.8%; with sulfonamide compounds alone, 9.2%.

The present report is an analysis of 134 consecutive cases of meningococcic infections admitted to the Station Hospital, Fort McClellan, Alabama, since March 13, 1942. This series occurred in two separate epidemic waves. The first epidemic of 15 cases occurred between March 13 and May 6, 1942. The second epidemic which began on December 7, 1942, consisted of 119 cases, and includes all cases up to April 16, 1943.

The criteria for establishing a diagnosis of meningococcic meningitis were: (1) Clinical signs of involvement of the central nervous system, such as nuchal rigidity, abnormal neurologic reflexes, *e. g.*, Kernig and Brudzinski signs and state of orientation and consciousness. (2) Confirmatory laboratory evidence of meningitic spinal fluid changes, with bacteriologic smears and cultures.

Despite the fact that several patients in the first epidemic were critically ill, and admitted to the hospital in coma, no fatalities occurred. The second epidemic presented a definitely more virulent type of infection. These cases were characterized by a stormier clinical course, with rapid progression until adequately treated.

All cases were males in previous good health, varying in age from 18 to 44. Ten patients in our series were colored, and 124 white, of which 44% were between the ages of 21 and 30. (Fig. 1.)

FIG. 1.—CLINICAL AND LABORATORY FEATURES

I. Race:		V. Blood cultures:	
White	124	Positive	42
Colored	10	Negative	89
II. Age distribution:		Not taken	*3
18–20 yrs.	58	VI. Lumbar puncture:	
21–30 yrs.	59	Clear fluid	55
31–40 yrs.	15	Cloudy fluid	79
41–50 yrs.	2	VII. Sulfa drug used:	
III. Skin rash:		*A.* Sulfadiazine	109
Petechiæ	96	*B.* Sulfanilamide	3
Purpura	9	*C.* Sulfadiazine and Sulfanilamide	17
No eruption	29	*D.* Untreated	*5
IV. Orientation:		VIII. Results:	
Conscious	82	Recovered	125
Stuporous	31	Died	9
Comatose	21		
Total per cent mortality	6.7%		
Mortality of treated cases	3.1%		

* Fulminating cases which expired within 12 hours after admission. (See text.)

These cases were fairly well distributed among the various units except for a large body of seasoned negro troops in which only one case appeared. This apparent paradox can best be explained on the grounds that the former were green, raw recruits while the latter were trained, seasoned, conditioned troops in which the incidence of upper respiratory infections was at a minimum. The percentage of negroes among the unseasoned troops was relatively low.

The sulfa drug employed in this series was sulfadiazine, but sulfanilamide was used in some cases (v.i.). Neither sulfapyridine nor sulfathiazole was employed in this series because of their more frequent toxic manifestations and the poor excretion of sulfathiazole into the spinal fluid.

The dosage of sulfadiazine in this series was much larger than that ordinarily recommended. Our basic plan of dosage was as follows: initial dose, 8 gm., second dose (2 hours later), 5 gm.; third and fourth doses (at 4 hour intervals) 4 gm.; subsequent doses, 3 gm. every 4 hours. This régime was practically constant in the first 24 hours. The blood sulfadiazine level was determined 18 to 24 hours after therapy had been initiated and daily thereafter, the subsequent dosages of sulfadiazine being regulated accordingly. We regarded the optimal blood sulfadiazine concentration to be between 15 to 20 mg. per 100 cc. If the blood sulfadiazine failed to attain satisfactory levels after the 1st day of chemotherapy, the dosage was "stepped up" to 4 gm. for one or two doses and then decreased to what we considered a maintenance dose—2 gm. every 4 hours and, in some instances, 2 gm. alternating with 1 gm. every 4 hours. Daily blood sulfadiazine levels, clinical appearance of the patient and absence of toxic manifestations were our dosage criteria.

In severe or fulminating meningococcic infections the intravenous administration of a 5% solution of sodium sulfadiazine was the initial procedure, since in these cases, it was imperative to obtain the optimal blood sulfadiazine level rapidly. The same route was used in instances of persistent vomiting. The medication was prepared by dissolving 5.5 gm. of sodium sulfadiazine in 111 cc. of sterile distilled water. Glucose in saline was usually given intravenously just prior to, or following, this medication. Immediately following the administration of the sodium salt intravenously, the basic plan of dosage described above was instituted. In stuporous or unconscious patients the oral drug was administered by Levin tube.

Sulfanilamide was administered in smaller doses than sulfadiazine as the optimum blood concentration of the former was regarded to be 12 to 15 mg. The initial dose of sulfanilamide was 4 gm. repeated in 2 hours and followed by 1 gm. every 4 hours for the first 24 hours of therapy. Dosage was then augmented or diminished, depending upon the blood sulfanilamide level. One gram of sodium bicarbonate was given routinely with each dose of sulfanilamide because of the frequency of acidosis with this drug. The substitution of sulfanilamide was reserved for those cases with renal complications due to sulfadiazine in which the sulfadiazine therapy up to that time had been inadequate.

It was difficult to determine when sufficient sulfadiazine therapy had been administered. There was no set rule or standard on which discontinuance of the drug could be based. Maintenance of therapy was largely guided by several factors—primarily the clinical course of the patient as well as the blood count, and by the presence or absence of toxic drug reactions. Temperature in our experience was notoriously inaccurate and unreliable as a guide.

In our series chemotherapy was discontinued only in the presence of the following toxic manifestations: hemolytic anemia, oliguria, anuria, and hematuria. The remaining complications—drug fever, cyanosis and renal colic—were not believed to be adequate contraindications for further use of the drug. The occurrence of renal colic, however, made us cautious in the further use of sulfadiazine but in itself was not felt to be sufficient reason for discontinuing therapy. The urinary output and fluid intake were closely watched in all cases, to insure satisfactory renal excretion. Daily specimens of urine were examined to detect any signs of kidney damage.

Discussion. The purpose of this presentation is to demonstrate the effect of massive doses of sulfadiazine on the course and outcome of a series of 134 consecutive cases of meningococcic infection. The clinical and laboratory features of the total series are summarized in Figure 1. There were 97 cases of meningitis and 18 of meningococcemia confirmed by laboratory and bacteriologic evidence. In the remaining 19 cases of meningococcemia no organisms could be found on smear or culture, but sufficient clinical and laboratory data existed to substantiate the diagnosis.

We have utilized doses of sulfadiazine much greater than ordinarily employed. Initial oral doses of 8 gm. were employed as contrasted with ordinarily recommended doses of 4 to 5 gm. Subsequent doses for the first 24 hours ranged between 3 to 4 gm. every 4 hours. Our average total dose of sulfadiazine for the first 24 hours was 25 gm. During the second day of treatment, the average total quantity of drug given varied between 12 and 15 gm. but this was not constant and was increased if an optimum blood concentration of sulfadiazine was not reached. The total amounts of sulfadiazine given to individual patients fluctuated widely. In our series, the largest total dose employed was 245 gm. and the lowest total dose 50 gm.: average approximately 68 gm. Factors involving absorption of the drug from the intestinal tract and rapidity of renal excretion or elimination must be considered in explaining these wide variations. Moreover, the severity of the infection, duration or stage of the disease, time of institution of therapy, varied clinical response of the individual to the drug, age of the patient, and time of appearance of toxic drug reactions materially affected the total dosage administered.

Of the 134 consecutive cases of meningococcic infection reported in this paper, a total mortality of 9 cases (6.7%) for the entire group resulted. All fatal cases were autopsied. However, 5 of these cases were inadequately treated and died within 12 hours of admission. An analysis of these 5 cases showed that 2 were fulminating meningococ-

cemias with the Waterhouse-Friederichsen syndrome; 3 cases were meningitis, in 2 of which the *Micrococcus catarrhalis* was isolated both culturally and by sugar fermentation tests. These 5 cases, therefore, cannot be included in the group of treated cases. Adequate therapy was defined as treatment with sulfadiazine or sulfanilamide over a minimal period of 24 hours. Four fatalities occurred in the remaining 129 patients who received adequate therapy, a treated mortality rate of 3.1%. Three of these cases exhibited meningococcemia with the Waterhouse-Friederichsen syndrome and the 4th case had a meningococcic meningitis.

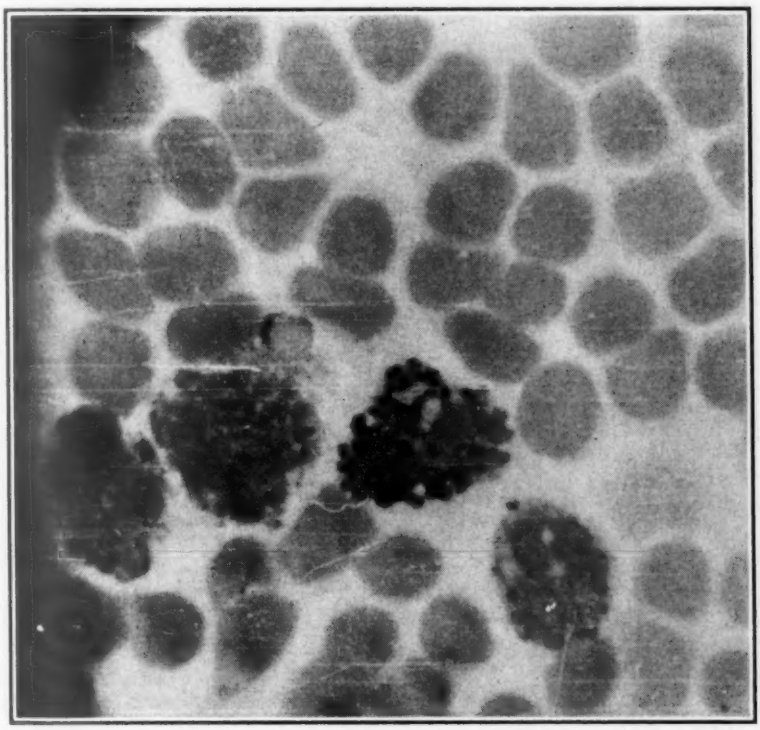

FIG. 2.—Smear of peripheral blood, demonstrating intracellular diplococci, and toxic degeneration of leukocytes. (Courtesy, Signal Corps, United States Army.) × 440.

The Waterhouse-Friederichsen syndrome accounted for 5 of the 9 deaths in our series. This syndrome is characterized by petechial or purpuric skin lesions and an overwhelming septicemia associated with massive adrenal hemorrhages. This condition is rare in adults: 70% of the recorded cases having been noted in children under 2 years of age. These 5 cases bring to 101 the total of reported cases in the literature up to the present time.[7] The survival periods of our cases of the Waterhouse-Friederichsen syndrome varied between 1 and 88 hours. The shortest-lived case (1 hour) showed Gram-negative diplo-

cocci in a routine blood smear (Fig. 2). On microscopic section, all revealed moderate to severe adrenal hemorrhages (Fig. 3).

The toxic effects of sulfanilamide and sulfadiazine encountered in this group are presented in Figures 4 and 5. The complications due to sulfanilamide therapy were in agreement with those reported by other observers. Eight out of 20 cases, (40%) treated with sulfanilamide had one or more toxic manifestations. Sulfanilamide was discontinued in the presence of hemolytic anemia and drug fever. We did not feel that the other drug reactions, acidosis and cyanosis, warranted discontinuance of chemotherapy. The 2 cases of hemolytic anemias encount-

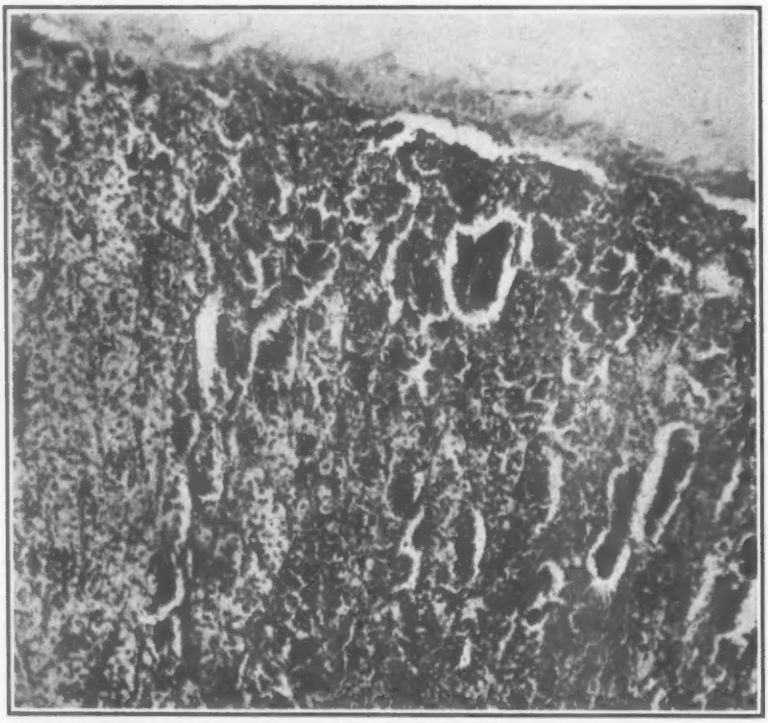

FIG. 3.—Section of adrenal gland showing massive interstitial hemorrhages in cortex and medulla, with few remaining islands of normal tissue. (Courtesy, Signal Corps, United States Army.) × 440.

ered were of a mild type and did not require blood transfusions. The temperature promptly dropped to normal in the drug fever group upon discontinuing the drug.

Drug toxicity with sulfadiazine was encountered more frequently than has been reported by other observers. The most serious toxic effects involved primarily the kidneys. In this series of cases complications of all types due to sulfadiazine reached a total of 28%. Of all complications 86% was renal in character. This is in marked contrast to the results in a series of over 300 cases of pneumonia treated with sulfadiazine cited by Ratish, Shackman and Bullowa[10] in which gross

hematuria occurred in only 3 cases (an incidence of 1%). No mention is made, however, of the frequency of microscopic hematuria. Finland, Peterson and Goodwin[5] found gross and microscopic hematuria in 5.2% of a series of 460 patients treated with sulfadiazine. Only a small group of these cases were of meningococcic infection, and the group, as a whole, required comparatively small doses of sulfadiazine.

We did not regard renal colic an indication for stopping sulfadiazine chemotherapy, as recommended by Keitzer and Campbell.[7] We continued the use of the drug cautiously, exercising great care in its administration. Six cases of renal colic were encountered, 2 of which developed oliguria, and 1, anuria. The remaining 3 cases showed no further evidence of renal irritation, despite the presence of renal colic.

Anuria is the most serious complication of sulfadiazine medication and fatalities have been reported.[6] One of our cases developed an anuria lasting 19 hours. This patient was treated conservatively. Fluids were forced by all routes and renal function was reëstablished.

Drug fever is an unusual complication of sulfadiazine medication, but the occurrence of sulfadiazine arthritis is even rarer. Due to our limited facilities we have not been able to make an exhaustive review of the literature on this latter subject. Drug fever with an incidence of 0.2% was reported by Finland *et al.*[5] in their series of 460 cases, but no cases of arthritis or arthralgia occurred in the same group. Arthritic manifestations attributable directly to sulfadiazine therapy occurred in 2 of our cases, in one of which drug fever was also present. The following case is cited because of its unusual interest and rarity.

Case Study. CASE 1 (Fig. 6). F. T. M., a 35 year old white male was admitted to the hospital on January 11, 1943, in an unconscious state, because of chills, vomiting, headache, pains in the joints and soreness of the muscles of 24 hours duration. On examination, patient was comatose and the skin covered with petechiæ. He presented the classical signs of a meningitis with nuchal rigidity and positive Kernig and Brudzinski. The pharynx was not injected. No spleen was palpable. Temperature on admission was 101.6° F., pulse 132, respiration 28.

A lumbar puncture showed a cloudy fluid under increased pressure containing 14,800 white blood cells with 95% polymorphonuclears. Globulin was positive. Smear revealed many extra- and intracellular Gram-negative diplococci. Blood and spinal fluid cultures were negative. The white blood count was 36,550 cells with 89% polymorphonuclears. A Levin tube was passed and an initial dose of 8 gm. of sulfadiazine given. Within the first 24 hours, the patient received 25 gm. of sulfadiazine orally. On the 2d day patient regained consciousness and was much improved, the temperature falling to 100° F., and pulse to 98. His only complaint was severe headache. The first blood sulfadiazine level 24 hours after sulfonamide therapy was reported 5.6 mg. In spite of large doses of sulfadiazine, the blood concentration of the drug rose slowly and reached 9.9 mg., 48 hours after chemotherapy was inaugurated. Subsequent sulfadiazine levels were no higher than 13.1 mg. reported on the 5th day. Pains in the right elbow and wrist first appeared on the 6th day, and these joints were found to be swollen, tender, painful and hot. The temperature fluctuated between 101° and 102° F. The joints were treated conservatively and large doses of acetysalicylic acid given with only slight relief. The sulfadiazine was continued and on the 7th day the left elbow and left wrist flared up similarly. Temperature persisted at 100.4° F. The left knee became painful and swollen on the 8th day. A sulfadiazine

level of 9.8 mg. and blood count of 15,150 white blood cells with a normal differential were reported on the 8th day. Joint symptoms persisted and, because of this, sulfadiazine was stopped on the 13th day. Within 36 hours after discontinuance of the drug, all joint manifestations subsided and on the 16th day remittance of the fever occurred.

Comment. The spectacular improvement in this patient after all drug therapy had ceased—the disappearance of joint symptomatology and subsidence of fever—made it plausible to attribute these effects to drug therapy. Since drug fever occurs infrequently with sulfadiazine, and joint manifestations are still rarer, the problem of differential diagnosis between drug idiosyncrasy and an occurrence of a new meningococcic focus localized in the joints was a difficult one.

The evidence for sulfadiazine arthritis was as follows: first, the appearance of migratory arthritis in spite of 6 days of massive sulfadiazine therapy; second, a falling white blood count; and third, the prompt and dramatic subsidence of fever and arthritis following discontinuance of the drug. Notwithstanding these symptoms it was thought best to err on the side of conservatism and to continue the administration of the drug, since it is well known that meningococcic infections are notorious in their recurrence after inadequate sulfonamide therapy.

FIG. 4.—INCIDENCE OF TOXIC MANIFESTATIONS OCCURRING IN 134 CASES OF MENINGOCOCCIC INFECTIONS

Sulfanilamide		Sulfadiazine	
Complication	Incidence	Complication	Incidence
Acidosis	1	Drug fever	3
Drug fever	3	Arthritis	2
Cyanosis	4	Anuria	1
Hemolytic anemia	2	Oliguria	4
Leukopenia	0	Hematuria:	
		(*a*) Microscopic	17
		(*b*) Gross	11
		Renal colic	6
Total cases	*8	Total cases	*36

* Several cases had more than one complication.

It is noteworthy to add that the patient gave an additional history of having sustained a fall, injuring both elbows, both wrists and his left knee, 1 week prior to his present illness. This brought up the interesting question of whether previously traumatized joints are more prone to sulfadiazine arthritis than normal joints. In one other case in our series an arthritic complication of a previously injured joint occurred under sulfadiazine therapy. The second patient with joint involvement injured his right knee while playing football 4 years prior to his present illness, and a diagnosis of bursitis was made. During the course of his meningococcic infection and on the 6th day of sulfadiazine administration, this traumatized knee became red and swollen. The knee signs and symptoms promptly subsided when the sulfadiazine was stopped. No other joints were involved.

Another important point which this case aptly illustrates is the disproportion between administration of large doses of sulfadiazine and

low blood concentrations of the drug. The highest blood level recorded in this case was 13.1 mg. on the 5th day, with levels varying between 4.4 and 13.1 mg. Nevertheless, low blood sulfadiazine concentrations do not militate against clinical recovery. The exact rôle of blood sulfadiazine levels as a factor in clinical improvement and cure is difficult to assay. Our experience has taught us that certain cases with satisfactory blood levels have done poorly while other cases with low blood concentration have had a remarkably smooth course. Low blood sulfonamide levels are not inconsistent with complete clinical cure, and the explanation may lie in the delicate balance existing between the rate of absorption of the sulfa drug from the bowel and the rate of elimination from the kidneys. It must be assumed in this patient that the relatively low blood concentration was due to either slow absorption from the intestinal tract, rapid renal excretion, or to diffusion and storage of the drug in the tissues. Slow reabsorption from such a tissue reservoir might well explain the continued elevation of sulfadiazine blood levels after cessation of therapy, which has been observed in some instances.

Summary. We have described in this paper our clinical experiences in treating 134 consecutive cases of meningococcic infections with massive doses of sulfadiazine and sulfanilamide. The total mortality for the entire series, treated and untreated, was 9 cases (6.7%). In our treated cases there were 4 deaths (a mortality rate of 3.1%). Analysis of our fatalities showed: (*a*) 5 cases (3 treated and 2 untreated) clinically and on postmortem exhibited the Waterhouse-Friederichsen syndrome; (*b*) 4 cases of meningitis, 1 treated and 3 untreated, which were proven on postmortem examination.

Although the diagnosis in a certain number of our cases could not be verified bacteriologically, sufficient clinical evidence in conjunction with laboratory data confirmed the clinical impression of a meningococcic infection.

FIG. 5.—THE INCIDENCE OF TOXIC MANIFESTATIONS AS RELATED TO THE MODE OF SULFADIAZINE ADMINISTRATION

Critical analysis of sulfadiazine	No. of cases	Per cent
I. Number of cases of meningococcic infection	134	
II. Number of cases receiving sulfadiazine	128	
(*a*) Oral sulfadiazine	82	64
(*b*) Intravenous and oral sulfadiazine	46	36
III. Number of cases having sulfadiazine complications—all types regardless of mode of administration	36	28
(*a*) Oral sulfadiazine complications	23	28
(*b*) Intravenous and oral sulfadiazine	13	28
IV. Number of cases having sulfadiazine renal complications—all types regardless of mode of administration	31	24
(*a*) Renal complications—oral sulfadiazine	19	23
(*b*) Renal complications—intravenous and oral sulfadiazine	12	26

The incidence of toxic drug manifestations in our series was higher than that usually reported. It is felt that this was due to the massive doses of the sulfonamide utilized. In our series, sulfadiazine was responsible for toxic reactions in 36 cases (28% incidence). Of this

group, 31 cases (86%) had some form of renal complication, exclusive of renal colic (Fig. 5). One case of sulfadiazine anuria was briefly mentioned. Two cases of sulfadiazine arthritis were discussed and evidence presented substantiating this diagnosis, with one illustrative case history.

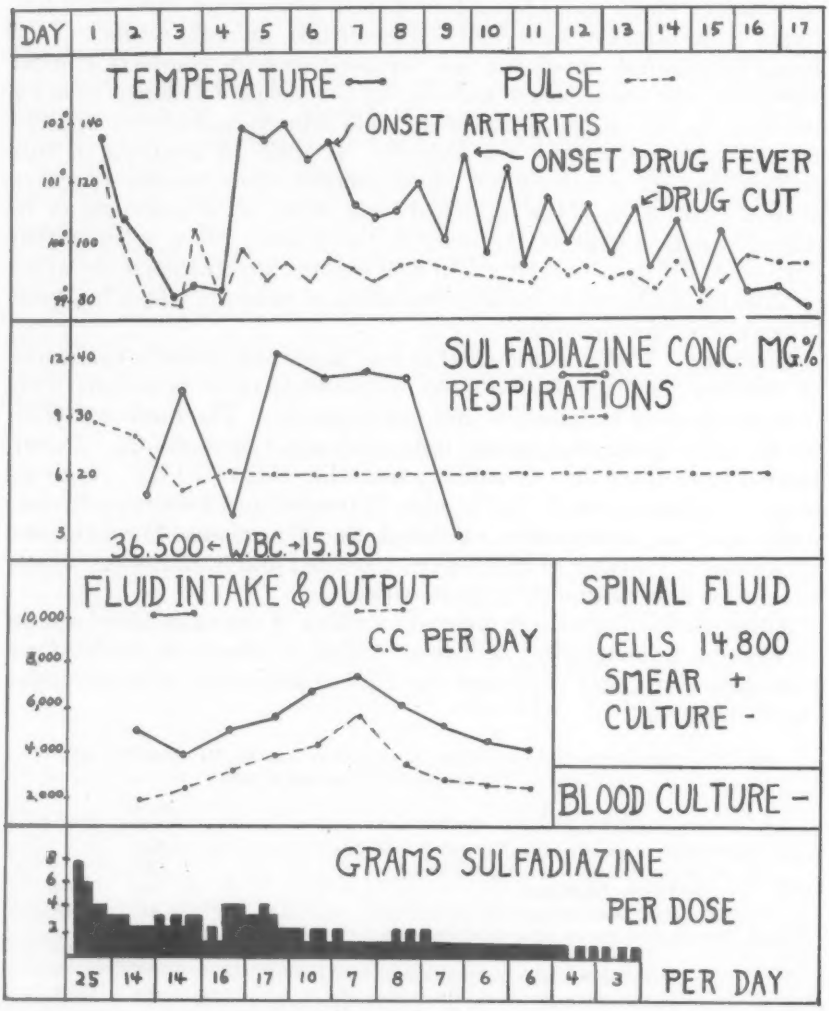

FIG. 6.—Case 1.

Conclusions. 1. From the results obtained in the treatment of 134 consecutive cases of meningococcic infections, we recommend the use of massive doses of sulfadiazine.

2. Our experience indicates that in the more seriously ill or moribund cases a satisfactory blood level is attained with difficulty with oral therapy. We feel that in these patients initial intravenous therapy should be used to complement oral medication.

3. The occurrence of 5 cases of the Waterhouse-Friederichsen syndrome in this group of patients indicates that it may be more frequent than hitherto reported in the literature.

4. An unusually high incidence of toxic manifestations due to sulfadiazine was encountered in our series of cases. However, our treated mortality rate of only 3.1% and no fatalities or residua which might be ascribed to the drug, justified the use of massive doses of sulfadiazine. Careful observation and supervision is absolutely essential in such massive therapy to prevent any undesirable consequences due to drug reactions.

5. Suitable evidence and data have been presented for the existence of sulfadiazine arthritis as a clinical entity.

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IDIOPATHIC HYPOPROTHROMBINEMIA

By Franklin D. Murphy, M.D.
and
John K. Clark, M.D.
Philadelphia, Pa.

(From the Department of Medicine of the Hospital of the University of Pennsylvania)

The development of accurate methods for the determination of plasma prothrombin[6a,12] has led in recent years to a much clearer understanding of various hemorrhagic diseases so that 2 cases, formerly regarded as atypical hemophilia, have been shown to have suffered from an idiopathic hypoprothrombinemia.[1,8] We have lately had the opportunity to study a patient with an obscure hemorrhagic diathesis in whom the most constant and outstanding laboratory finding was hypoprothrombinemia, totally resistant to vitamin K therapy and lacking adequate explanation. This case would probably have been classed in the past as pseudohemophilia as his bleeding time was frequently prolonged, venous coagulation time always normal, platelet count normal and there was a history of abnormal bleeding in a sister. However, he shared the cardinal feature of both previously reported cases of idiopathic hypoprothrombinemia and there seems little doubt

that the deficiency of this factor was responsible for his hemorrhagic tendency.

Case Report. R. K., an 18 year old white male of Pennsylvania Dutch stock, was admitted to the medical wards of the Hospital of the University of Pennsylvania on 3/10/43 with the chief complaint of persistent bleeding from the gums and epistaxis of 3 months' duration.

The history began at the age of 4 when, after fracturing his nose in a fall, he developed profuse epistaxis which was controlled with difficulty. Thereafter, until the age of 10, nosebleeds, of varying degrees of severity, occurred about once a month. From the age of 10 years until his admission to this hospital epistaxis had become more frequent and severe, occurring 2 to 3 times per month, and in addition bleeding from the gum margins had first made its appearance. In October, 1939, he was admitted to the Easton Hospital, Easton, Pa., complaining of hematuria. Blood studies at this time revealed bleeding time 1½ minutes, coagulation time 3 minutes, prompt clot retraction and platelets 113,600.

Following severe epistaxis, the patient was admitted to the Allentown Hospital, Allentown, Pa., in January, 1943, where studies revealed a prolonged prothrombin time. He was then sent to this hospital for additional observation.

The past medical history was non-contributory except for the above-mentioned hemorrhagic tendency.

Family history, including investigation of father, mother, sibling, maternal and paternal grandparents, aunts, uncles and cousins revealed nothing of a contributory nature except for fatal bleeding in the patient's only sibling, a sister. The history of this girl was kindly made available by Dr. Leslie Freeman of Easton, Pa., and is as follows: B. K., a young white female, aged 4, was admitted to the Easton Hospital in June, 1931, with chief complaints of epistaxis and abdominal pain. The patient had been afflicted with nasal bleeding from the age of 3 years. Physical examination revealed an anemic child with tenderness around the umbilicus and hemorrhagic areas over the abdomen. Blood platelet count was normal on two occasions, bleeding time was 15 minutes and coagulation time 4 minutes. After several transfusions, the parents signed the child's release from the hospital. She was readmitted in October, 1932, with a history of profuse epistaxis of 3 weeks' duration. Examination revealed a palpable spleen. Laboratory studies showed severe anemia, normal platelet count and normal bleeding and clotting time. The patient died 14 hours after admission and the necropsy was reported as showing "splenic anemia."

Occupational and social history of R. K. was unessential, and there was no history of undue exposure to known toxic materials.

Physical examination on admission revealed an intelligent, alert but somewhat apprehensive young white male. Temperature was 98.3° F., pulse 73, blood pressure 100/60, height 66 inches, weight 104 pounds. The significant physical findings included evidence of recent bleeding in the nose, marked dental caries, a red, fissured tongue, congested and bleeding gums, palpable spleen which descended 1 to 2 cm. below the left costal margin with deep inspiration and bilateral sustained ankle clonus, not regarded as significant by a neurologic consultant. There was no evidence of jaundice and no petechiæ or telangiectases could be found by careful examination, including nasoscopic study. The liver could not be felt and there was no evident lymphadenopathy. Ophthalmoscopic examination revealed normal fundi.

Laboratory Studies. *Blood:* R.B.C. 4,600,000, Hb. 77%, reticulocytes 7%, W.B.C. 7850, neutrophils 70%, lymphocytes 19%, monocytes 7%, eosinophils 4%. Red blood cell fragility tests showed hemolysis beginning at 0.45 and ending at 0.32 and on repetition comparable results were obtained. Erythrocyte sedimentation rate was 11 mm. per hour by the modified Rourke-Ernstene method. Figures for bleeding time, coagulation time, prothrombin time and blood platelet counts are shown in Table 1. Clot retraction was prompt and good and platelet morphology normal. Kolmer

and Klein tests were negative. Blood type was A and Rh+. *Urine:* Specific gravity was found from 1.012 to 1.028. There was no albumin or sugar and the urinary sediment was always quite normal. *Blood chemistry:* Blood urea nitrogen was 9 and 7 mg. per 100 ml., serum proteins 6.1 and 6 gm. per 100 ml. with the albumin 4.6 and globulin 1.4 gm. per 100 ml. giving an A/G ratio of 3.2. Serum calcium was 9.8 and 10.3 mg. per 100 ml., serum cholesterol 229 and 147 mg. per 100 ml., blood uric acid 4.8 and 3.7 mg. per 100 ml. and serum bilirubin less than 0.5 mg. per 100 ml. on two occasions. Plasma vitamin C level was 1.5 mg. per 100 ml. and plasma fibrinogen 331 mg. per 100 ml., both values being within normal limits. Glucose tolerance test showed a fasting level of 60, ½ hr. level 115, 1 hr. level 75 and a 2 hr. level of 90 mg. per 100 ml. *Liver function studies:* Cephalin-cholesterol flocculation test showed a 5 mm. flocculum in 48 hours. Hippuric acid excretion was 1.22 gm. in 1 hour and bromsulphalein test revealed 5% retention of dye after 30 minutes. *Roentgen ray* films of chest were normal. Examinations of the gastro-intestinal tract were normal, except for a roundworm seen on one occasion. Body section roentgenograph of the upper abdomen very successfully revealed a slightly enlarged spleen but normal sized liver. Roentgen ray of skull and urogram were within normal limits. Dental Roentgen rays showed numerous carious teeth. *Miscellaneous studies;* Two Gothlin indices as well as the usual tourniquet test were negative. Electrocardiogram was normal. Examination of bone marrow aspiration revealed normal inhabitants in the usual number. Urea clearance was 75% of average normal function. Microscopic examination of the capillaries of the nail bed revealed definitely abnormal and bizarre forms.

Because of the hypoprothrombinemia the patient was given several naphthoquinone derivatives by different routes. On 3/17/43, he was given over a 24 hour period a continuous venoclysis of 3000 cc. of 5% glucose to which was added 20 mg. of Synkavite (2-methyl 1, 4 naphthohydroquinone—diphosphoric acid ester tetrasodium salt). Before the start of the venoclysis the prothrombin time was 70 seconds and at the conclusion the figure was 80 seconds (normal control 16 seconds). From 4/4/43 to 5/7/43 the patient received Proklot (2-methyl 1, 4 naphthoquinone) mg. 1 p.o. with bile salts and Synkavite mg. 10 i.m. daily. As may be seen from Table 1, the plasma prothrombin level was not significantly affected. It was also clear that these preparations of vitamin K had no effect upon the bleeding. From 4/19/43 to 5/7/43, the patient was given Hesperiden mg. 250 t.i.d. without effect. On 5/12/43 administration of adrenal cortical extract was tried. Prothrombin time before administration was 64 seconds. Cortin (Upjohn), 20 cc., was given i.m. and 5 hours later prothrombin time was 58 seconds. Then an additional 10 cc. were administered, 5 cc. i.m. and 5 cc. i.v. Five hours after the second and 10 hours after the first dose, the prothrombin time was 54 seconds, a non-significant change. The only adequate hemostatic for this patient was whole blood and it was in every instance quite effective in stopping the bleeding. As little as 100 cc. of blood stopped hemorrhage within an hour and prevented bleeding for from 1 to 3 days. Administration of 500 cc. of blood usually inhibited bleeding for about 1 week.

Shortly after admission it was discovered that the patient was infested with *Ascaris lumbricoides*. Hexylresorcinol therapy was efficacious. The prothrombin levels were in no wise affected by the removal of the worms. During the hospital stay, 6 teeth were extracted and by means of frequent small transfusions, bleeding was kept to a minimum. Beef thrombin, applied locally to the socket failed to inhibit bleeding, an observation also made when it was applied to the bleeding gums.

Recently, it has been reported by Link *et al.*[4] and Rapoport *et al.*[7] that administration of salicylates may result in hypoprothrombinemia in experimental animals and man. However, in our patient both complete avoidance of salicylates (of which he had been getting an occasional 10 gr. dose) for a period of 3 weeks as well as administration of large doses for several days were without effect on his plasma prothrombin.

Lacking precedent, the question of future therapy and prognosis was difficult to answer. Temporarily a hospital job has been arranged for the boy so that transfusions may be made available as needed.

Special Hematologic Studies. Studies of the blood coagulability of the patient and his family are seen in Table 1. Our patient showed an invariably prolonged plasma prothrombin time by the Quick method,[6b]) unaffected by vitamin K therapy or blood transfusion. The bleeding time was prolonged on several occasions but the coagulation time, clot retraction and platelet count were always within normal limits. The father had a slightly prolonged prothrombin time, probably of no significance, and normal bleeding time, coagulation time and platelet count. The mother's studies were quite normal. Studies done at the Easton Hospital in 1931 on the sister's blood showed increased bleeding time, normal coagulation time and platelet count. No prothrombin determination was done.

TABLE 1.—SOME BLOOD DATA IN THE K FAMILY

Date	Prothrombin time (Quick)* (sec.)	Bleeding time (Duke) (min.)	Coagulation time (Lee-White) (min.)	Platelet count (in thousands per c.mm.)
	Patient R. K.			
3/11/43	68	12	7	280
3/17/43	70	..	10	234
3/18/43	80			
3/22/43	70	4	..	358
3/23/43	79			
3/24/43	..	6	7	
3/31/43	92			
4/ 2/43	80			
4/ 5/43	60			
4/ 6/43	..	1½	..	234
4/17/43	85	2½		
4/20/43	..	5½	10	254
4/21/43	72			
4/28/43	90			
5/17/43	100	15	10	291
	Father of R. K.			
4/18/43	19	2	9	280
	Mother of R. K.			
4/ 8/43	16	1½	8	256
	Sister of R. K.†			
5/12/31	..	15	4	219
5/23/31	..	..	..	297

* Normal control—16 seconds.
† Studies done at Easton Hospital, Easton, Pa.

In order to check for the presence of antiprothrombin and antithrombin our patient's plasma was mixed with normal plasma in equal parts. The prothrombin time of the normal plasma was 16 seconds and that of the mixture was 18 seconds. This prolongation of the prothrombin time was exactly the same as that obtained after mixing the normal plasma with a prothrombin-free fluid such as 0.85% NaCl solution and was thought to demonstrate the absence of an excess of antiprothrombin or antithrombin.

To rule out the possibility that an excess of heparin was a factor, protamine was mixed with R. K.'s plasma in concentrations of 1/25, 1/50, and 1/75 mg. of protamine to 1 cc. of plasma. These mixtures caused pro ongation of the prothrombin time but only to a degree to be expected from dilution with saline solution. In addition, the patient was given 40 mg. of protamine intravenously. Before injection, the prothrombin time was 92 seconds, and 30 minutes after injection it was 65 seconds. No further attempt was made to give the patient protamine because of the development of a marked sensitivity

to this protein. The significance of the finding is difficult to interpret but it may be stated that the normal coagulation time associated with marked hypoprothrombinemia speaks strongly against an excess of heparin as being the cause of the low plasma prothrombin and bleeding in this patient.

It was felt necessary to check the prothrombin along the lines suggested by Warner, Brinkhous and Smith.[12] Accordingly such a procedure was carried out by Miss Lillian Panzer on 5/5/43. After incubating the fibrinogen-free plasma of a normal control with thromboplastin and calcium for 90 seconds, a solution of fibrinogen was added and a clot formed in 14 seconds at a plasma dilution of 8 to 1 while R. K.'s plasma, treated in the same fashion required 48 seconds to clot in a dilution of only 2 to 1. The results were taken to mean that the prothrombin content of R. K.'s plasma was quantitatively low and that the fault was not merely in the conversion of prothrombin to thrombin.

Since the plasma fibrinogen of one of the reported cases of idiopathic hypoprothrombinemia was apparently abnormally resistant to the action of thrombin,[8] it was thought best to check R. K. for such an abnormality by the addition of fibrinogen preparations to his plasma. On 4/21/43 a solution of fibrinogen, freshly prepared from normal blood was mixed in equal parts with R. K.'s plasma. Before the addition of fibrinogen, the prothrombin time was 72 seconds while that of the mixture was 45 seconds. This was repeated on 4/28/43 and again the prothrombin time was reduced, this time from 90 to 65 seconds. A prothrombin determination done on the fibrinogen solution showed no clot in 900 seconds which indicated that the solution was practically free of prothrombin. An additional study involving the addition of deprothrombinized normal plasma, prepared by shaking with colloidal aluminum hydroxide, to R. K.'s plasma showed no reduction in the prothrombin time. This result was difficult to interpret. Nevertheless, it seemed that there was definitely suggestive evidence of some type of qualitative defect of R. K.'s plasma fibrinogen.

Another curious finding was that dilution of R. K.'s plasma with equal parts of saline only increased the prothrombin time from 79 to 94 seconds, definitely less than would be expected. This observation was also made in a previously reported case of idiopathic hypoprothrombinemia.[8]

Discussion. In 1941, Rhoads and Fitz-Hugh[8] reported the case of a young white male, previously diagnosed as atypical hemophilia, in whom the discovery of marked hypoprothrombinemia, resistant to the administration of vitamin K, required that the diagnosis be changed to idiopathic hypoprothrombinemia. The patient died as a result of central nervous system hemorrhage and examination of the liver revealed some atrophy of liver columns but the changes were considered too slight to have been the cause of the low plasma prothrombin. The case of Beard, quoted by Quick,[1] was similar in many respects to that of Rhoads and Fitz-Hugh but in spite of the fact that the bleeding continued in this case, the hypoprothrombinemia responded favorably to vitamin K therapy. Our patient had certain important features in common with the patient of Rhoads and Fitz-Hugh. Both had marked hypoprothrombinemia unresponsive to the administration of various preparations of vitamin K given by various routes. The platelet count of both was never low enough to account for abnormal bleeding. Hemorrhage in both had started early in life and their bleeding times were frequently prolonged. Special studies revealed that the plasma fibrinogen of both patients was qualitatively defective although this was shown less well in our patient. There were, however, definite differences between the 2 cases. The coagulation time of R. K. was

always normal, clot retraction good, bleeding usually from nose or gum margins and there was a familial history of bleeding in a female sibling. The patient of Rhoads and Fitz-Hugh, on the other hand, had a prolonged coagulation time, poor clot retraction, hemarthrosis was common and there was no familial history of abnormal bleeding. Despite these differences, it seems fair to reëmphasize the fact that the bleeding in both patients was probably due to unexplained hypoprothrombinemia and that they, therefore, deserve to be classified in the same general group which as yet is not understood well enough to stand sub-classification.

Another significant point is that R. K., except for the hypoprothrombinemia, seemed to fit quite well into that rather ill-defined disease entity called pseudohemophilia, and in fact such would have probably been his diagnosis had not the prolonged prothrombin time been discovered. Increased but variable bleeding time, normal coagulation time and normal platelet count, found in R. K., are characteristically present in pseudohemophilia. Abnormal capillaries, seen in the nail-bed of our patient, have been shown by Macfarlane[5] to be present in pseudohemophilia. The type of bleeding, negative tourniquet test and even the slightly enlarged spleen are compatible with a diagnosis of pseudohemophilia. The bisexual hereditary and familial history, required for a definite diagnosis of this disease, is represented in our patient in part by a fatal hemorrhagic disease in the only sibling, a sister. A review of the literature reveals that those few cases of pseudohemophilia[3,9,10,11] in which plasma prothrombin was apparently determined by accurate methods, had normal or only slightly increased prothrombin times. There is, however, distinct laboratory and clinical variability among the cases of hemorrhagic diathesis which have been grouped together as pseudohemophilia so that this disease is still not sharply defined.[2] Since the plasma prothrombin has been studied by modern methods in only a few cases of this disease, it seems possible that prothrombin determinations on a large number of these patients might demonstrate a lack of this factor as the cause of bleeding in certain cases of pseudohemophilia.

The cause of the hypoprothrombinemia in our patient can be only speculative. There are 5 main situations in which a low prothrombin is found,[6b] namely: deficiency of vitamin K; inadequate absorption of vitamin K from the gastro-intestinal tract; poor utilization of vitamin K by the liver; action of toxins (dicoumarin); and idiopathic. The first 2 situations were shown not to be present in our patient as there was no significant response of his plasma prothrombin to the parenteral or oral administration of vitamin K. If this patient's marked hypoprothrombinemia were due to poor hepatic utilization of vitamin K on the basis of liver damage, one might reasonably expect definitely abnormal results in some or all of the tests for liver function which was not the case with R. K. as studies of hepatic function, taken as a whole were not abnormal. Furthermore, liver size was normal and the patient did not present the clinical picture usually associated with liver disease of a degree required to give this severe and resistant

hypoprothrombinemia. Nevertheless, the poor sensitivity of the liver function tests in use today does not allow the complete elimination of liver damage as the cause for this patient's low plasma prothrombin. One could hypothesize, however, that R. K.'s primary fault was a specific inability of his liver to synthesize prothrombin, occurring in the presence of relatively normal liver function in other respects and possibly familial in character.

As to toxic causes of hypoprothrombinemia, it is interesting to note the similarity of R. K. to patients who have received overdoses of dicoumarin. However, we were never able to demonstrate any evidence that a toxin was influencing R. K.'s prothrombin and this seems further borne out by the fact that changes in diet and environment were without effect.

It appears then that the term idiopathic is needed to describe this case and that the cause of the hypoprothrombinemia cannot be definitely stated.

Summary. A case of apparent idiopathic hypoprothrombinemia has been presented which was in most respects comparable to 1 of the 2 previously reported cases of this condition.

An additional feature was the marked similarity of our patient's disease to pseudohemophilia.

This case presented many points of similarity to patients receiving overdoses of dicoumarin, but no adequate cause for the hypoprothrombinemia was demonstrated.

Since this paper was submitted for publication, another case has been reported which in most respects is similar to our case.[2a]

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JOHN H. MUSSER, M.D.
PROFESSOR OF MEDICINE, TULANE UNIVERSITY OF LOUISIANA, NEW ORLEANS, LA.

OXYGEN POISONING*

By William C. Stadie, M.D.
JOHN HERR MUSSER PROFESSOR OF RESEARCH MEDICINE

Benjamin C. Riggs, M.D.
INSTRUCTOR

AND

Niels Haugaard, A.B.
RESEARCH ASSISTANT

(From the John Herr Musser Department of Research Medicine, University of Pennsylvania)

Introduction. Lavoisier had no sooner discovered oxygen and its significance to life than experimenters began to subject animals to concentrations of oxygen greater than that in the air. Indeed, Lavoisier himself with Seguin[118] established the fact, since abundantly confirmed, that inhalation of 1 atmosphere of oxygen does not alter the oxidative metabolism. Early, administration of oxygen to the sick made a strong appeal to the imagination—both lay and professional. Rational oxygen therapy slowly evolved and was put upon a sound basis by the introduction of the arterial puncture—to determine hemoglobin unsaturation, and the oxygen chamber, tent, and mask—to assure efficient administration. The improved technical production of cheap, pure oxygen was, of course, an important contributing factor in this advance.

Two other fields of human activity were meanwhile increasing, the one old, the other new: namely, deep-sea diving and flying. Oxygen inhalation at greater than normal pressures assumed importance in these. Paul Bert, devoting years to the study of physiologic reactions in the first of these fields, embodied his findings in his classic, *La Pression Barometrique* (1878).[40] For the first time he revealed that oxygen plays a dual rôle. On the one hand—at low pressures—it sustains life; on the other—at high pressures—it kills. "One finds clearly demonstrated," he said, "the apparently paradoxical result that under the influence of very high oxygenation

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of the blood, the tissues oxidize less, organic combustions diminish in energy, production of CO_2, excretion of urine, the destruction of sugar in the blood are diminished, and that as a result temperature falls." (Reviewers' translation.)

Thus began the subject of oxygen poisoning. Literature has accumulated; at first sight scanty, but upon search, surprisingly extensive. Various points of view were emphasized—deep-sea diving, aviation, microorganisms, new growths, enzymes, blood gas transport, therapy. Some wrote a paper or two; others devoted years to the subject. In his Harvey Lecture, Behnke[30] enumerated the fruits of these labors: Increased knowledge of alterations in the gaseous environment of man, increased efficiency of the diver and caisson worker, clarification of puzzling phenomena of flight, and greater possibility of salvage work on sunken wrecks.

There are three types of situations involving possible oxygen poisoning: (1) At high altitudes, as in flying, where supplemental oxygen is given to counteract the insufficiency of the air. Since the ambient pressure is low, there need be no excess oxygen pressure;* hence, as Armstrong stated,[12] "There have been no harmful effects from the use of oxygen in aviation except some of a relatively minor nature." (2) In deep-sea diving, in which toxic pressures of oxygen may be quickly reached. It is the dream of those interested that an understanding of the mechanism of oxygen poisoning will enable man to descend to depths limited by the strength of materials rather than his physiology. (3) In disease, particularly pulmonary and cardiac, producing insufficient saturation of blood hemoglobin, alleviated by oxygen administration. Poisoning may arise here if the safe dose is exceeded.

The first exposition of the subject was that of Bert. Subsequently Hill[95] devoted a portion of his book "Caisson Disease" to partial review and discussion of oxygen poisoning. Apparently there has been no exhaustive review. The authors present here a discussion of significant papers on the subject.

Bert, the discoverer of the phenomenon called it, according to the Hitchcock[40] translation, oxygen poisoning. By this is meant any variation from the normal structure or function attributable to the action of oxygen which produces deleterious effects.

Symptomatology. Excess oxygen produces characteristic symptoms and signs, the rapidity of onset and severity of which vary with the partial pressure of oxygen. While there seems to be general agreement that oxygen toxicity constitutes a clinical entity, there is less agreement on the incidence of specific symptoms, with the exception of those referable to the central nervous system.

1. *Nervous System.* Bert[40] studied the effects of increased air pressure on several animals, including birds, dogs and cold-blooded species. The outstanding symptom in all cases was convulsion, characterized by tetanus and opisthotonus, said to resemble strychnine poisoning. Later investigators described similar convulsions at various pressures in several species. Bornstein and Stroink[48] found clonic spasms developed in rats at 8 atmospheres of oxygen, Hill[95] and Shilling and Adams[152] described convulsions in several mammalian species at 2 to 5 atmospheres. Libbrecht and Massart[120] observed convulsions in mice at 4 atmospheres, and Hederer and Andre[91] found convulsions the presenting symptom in rabbits at from

* All pressures of oxygen will be stated in terms of atmospheres absolute. Ordinarily, when administering oxygen, it is sufficient to indicate the concentration by stating the per cent. At higher pressures, however, such a designation becomes confusing.

0.8 to 12 atmospheres of oxygen. Behnke *et al.*[36] subjected dogs to 4 atmospheres of oxygen for 2 to 4 hours, and found that 2 out of 9 developed convulsions, which were described as respiratory in type. Later, Shaw, Behnke and Messer[151] stated that "the symptoms of acute oxygen poisoning" (at 4 atmospheres of oxygen) "which are most apparent are the changes in blood pressure" (see below) "and the convulsive seizures of the head and neck." Although generalized convulsions have not been as frequently observed in man as in other species, Behnke stated[30] that "essentially oxygen induces transient idiopathic epilepsy in the apparently normal individual." More specifically, Bornstein and Stroink,[48] while finding no symptoms in man at rest for 45 minutes in 2 atmospheres of oxygen, observed clonic spasms of the legs with hyperactive patellar reflexes when the subject was working on a bicycle. Behnke, Johnson, Poppen and Motley[34] studied the effect upon man of 1 to 4 atmospheres of oxygen, and described the symptoms as all referable to the nervous system; these included more or less generalized convulsions, syncope, and loss of coördination and attention. Thomson[164] observed 2 naval officers under 4 atmospheres of oxygen; 1 of these developed facial twitches in 16 minutes and the other, a tremor of the lips. When the second subject was returned to air at normal pressure, clonic seizures were followed by unconsciousness. The possibility that the symptoms in the second case were due to aëro-embolism cannot be excluded. (See section on Aëro-embolism.) Behnke, Forbes and Motley[33] studied the effects of oxygen at 4 atmospheres on men. They measured visual changes to be described later, but also observed dizziness, stupefaction and a "sense of impending collapse." Substitution of air by removal of the mask was followed by recovery and a sense of exhilaration. Becker-Freysang and Clamann[27] subjected themselves to 0.9 atmosphere of oxygen for 60 hours, and after the first 24 noticed paresthesias of the fingers. Behnke[28] described true epileptiform convulsions developing 45 minutes after subjection to 4 atmospheres of oxygen. In a review of observations pertinent to submarine salvage work, Jenkinson[109] gave as characteristic symptoms twitching of the head and neck, convulsions and paresthesias. Moody and Howard[132] described convulsions in a child with pneumonia who was treated for 6 days in an oxygen tent with a concentration presumed to be over 0.7 atmosphere although it was not measured in this case.

The non-specificity, or general nature of the central nervous system response to high oxygen is suggested by the observations of Bean and Rottschafer[25] that the removal of the cortex, and even of the cerebrum in dogs had no appreciable effect upon the convulsions produced by 5 to 6 atmospheres of oxygen.

There has been only one piece of work done on the special senses, by Behnke, Forbes and Motley,[33] who found that 3 atmospheres of oxygen produced, among other effects, a reversible loss of visual acuity, contraction of the fields to the tubular type (reminiscent to the Reviewers, of that seen in advanced central nervous system lues) but with pupillary dilatation.

The mental effects of oxygen at high pressures were first described in detail by Hill and Phillips.[101] They associated personality types with the appearance of certain symptoms. For example, those divers showing overcontrol, suppressed fears and resentment of observation and direction, designated by the authors as the "philosophical type," were prone to show work failures, dizziness and claustrophobia at 7 to 8 atmospheres of air pressure (oxygen = 1.4 to 1.6 atmospheres). In contrast, the

"practical type" were relatively free of symptoms. Nitrogen narcosis as a contributing factor in these symptoms was not discussed by the authors. Previous to this, Binger and Faulkner[42] had found that rabbits and dogs became drowsy within 3 days, breathing 0.8 atmospheres of oxygen, although Paine, Lynn and Keys[137] later produced lethargy in dogs in 2 hours at 1 atmosphere of oxygen. Davidson,[77] working with 1 atmosphere of oxygen, found no effect upon reaction time in choice tests in 20 minutes, but Shilling and Willgrube,[154] in a highly quantitative report, showed a slowing in problem-solution, cross-out tests and light-to-touch reactions with increasing pressure. The amount of slowing was also related to the subject's mental ability. To eliminate possible confusion with nitrogen narcosis, they studied these effects with increasing time at a given pressure and with increasing experience, and found the mental slowing was reduced in each case. Haldane[90] distinguished the mental effects of high oxygen from those of high carbon dioxide and nitrogen in the period of recovery: in the former, there is marked terror, but in the latter two, there is calmness. Behnke[30] described "mental torpidity" as characteristic of oxygen poisoning. Although the effects of oxygen on the respiratory system will be separately considered, it should be mentioned here that Barach[15] found, in patients with a preëxisting chronic anoxia, that inhalation of 0.5 atmosphere of oxygen produced profound but reversible disturbances in mental functioning, consisting of deep sleep and stupor followed by coma and delirium. This finding supports Hill's statements[95] that coma and paralysis are frequent symptoms in animals.

Respiratory Symptoms. As the discussion on the disorders produced by high oxygen will demonstrate, the respiratory system is second only to the nervous system in its apparent susceptibility to the gas. The first symptom to be expected is dyspnea. While Hill[95] pointed out that the earlier authors, from the time of Lavoisier to 1912, described respiration under increased air pressure as variously increased and decreased in amplitude and frequency, there seems to have been little mention of dyspnea. Hill found this symptom dominant in several species of warm-blooded animals, and earlier in onset than the central nervous system symptoms. Binger, Faulkner and Moore[42] found that dyspnea and cyanosis were the dominant symptoms in rabbits and dogs exposed to 0.8 atmosphere of oxygen for longer than 3 days. (This is related to the pulmonary changes to be described later.) Most of the rats placed in 1 atmosphere of oxygen for 3 to 4 days by Boycott and Oakley[49] showed increasing dyspnea as the only symptom, and died apparently from dyspnea described as "explosive." Paine, Lynn and Keys[137] also found dyspnea the most important symptom in dogs placed in 0.9 to 1 atmosphere of oxygen; the time of development varied from 4 to 75 hours. Gesell[88] found essentially the same symptoms.

Passing from dyspnea to more general variations in respiratory effort, Achard and Leblanc[2] described the death of guinea pigs and rabbits in 0.8 atmosphere of oxygen as due to asphyxia following slowed and jerky respiration appearing in the second day. Smith, Heim, Thomson and Drinker[158] subjected rats to 4 atmospheres of air pressure (0.8 atmosphere of oxygen), and noted that on the 3rd day, the animals were acutely ill, showing hyperpnea and cyanosis. In connection with the convulsive seizures described by Shaw, Behnke and Messer above,[151] these authors further stated that the seizures are "essentially inspiratory convulsions and are not unlike those in certain types of asphyxia." The convulsive period was, in fact, "terminated by respiratory failure." Behnke[28] found

in healthy males after a 7-hour "tolerance period" in 1 atmosphere of oxygen, an increased respiratory depth. Finally, Paine, Keys and Lynn[137] described the death of dogs in 0.9 to 1 atmosphere of oxygen within 48 to 120 hours as occurring after a period of respiratory distress. Marshall and Rosenfeld[126] found in addition that the administration of oxygen will enhance the respiratory depression from various drugs to the point of apnea and respiratory failure. Although more important from a metabolic point of view, the measurement of respiratory minute volume also bears a relationship to respiratory symptoms. While Hill[95] could find little change under compressed air, Clark-Kennedy and Owen[72] reported a decrease in pulmonary ventilation when the oxygen was raised to only 0.26 atmosphere (far below the minimum concentration at which most authors report symptoms). On the other hand, Bean,[17,18] found an increased respiratory minute volume in dogs at 4 to 5 atmospheres of oxygen, and Bean and Haldi[24] confirmed this in dogs at 5 atmospheres, although there was no increase at 1 atmosphere. Bean and Rottschafer[25] also found an increase at 5 to 6 atmospheres in decorticate and decerebrate dogs. In man, however, Behnke, Johnson, Poppen and Motley[34] found no change in minute volume at pressures from 1 to 4 atmospheres. In fact, "the irritative effect of oxygen on the lungs was noted in only one subject" (out of 10) "on a single occasion," although an increase in respiratory rate was observed. The single occasion was at low pressure (1 atmosphere). Schwab, Fine and Mixter[148] found no change in respiration in patients breathing 1 atmosphere of oxygen for 3 hours. Similarly, Becker-Freysang and Clamann[27] could detect no consistent change in respiration in themselves at 0.9 atmosphere of oxygen for 60 hours, although at the end of the experiment one of the authors developed bronchopneumonia, presumably a result of the high oxygen tension. Vital capacity was decreased in 1 subject and unchanged in the other. In dogs subjected to 1 atmosphere of oxygen for 6 minutes, Watt, Dumke and Comroe[168] found a transient diminution in respiratory minute volume of 11 to 13%. After elimination of respiratory chemoreceptors by the denervation of the carotid and aortic bodies, however, there was no change, and sometimes an increase. The authors concluded that since the chemoreceptors are continuously activated by the usual degree of oxygen unsaturation of the blood, the decrease of this saturation by high concentrations of oxygen eliminates this activation, resulting in diminution in respiratory minute volume.

Two general conclusions seem warranted from the evidence given above. The first is that respiratory symptoms are dominant in the action of oxygen at 0.8 to 2 atmospheres, while central nervous system symptoms are dominant above this level. This may perhaps be because at high pressures, death due to central nervous system symptoms precedes the possible development of pulmonary symptoms. The second conclusion is that in any case oxygen appears to be less irritating to the pulmonary system in man than in other species. However, conclusions with respect to man must be guarded since data are limited.

Cardiovascular Symptoms. Because of the comparative paucity of data, it is convenient to group together the heart rate in the intact organism, the blood pressure and the vasomotor signs of pallor and flushing. Bert observed[40] that under 3 to 4 atmospheres of air pressure (oxygen = 0.6 to 0.8 atmosphere), the pulse rate was lowered, and there appeared to be an increase in arterial tension, although this was not measured. Hill[95] confirmed the results obtained by other early investigators who found no

appreciable change in either pulse rate or blood pressure. Parkinson[138] observed a definite decrease in pulse rate when men breathed oxygen through masks at atmospheric pressure for 30 minutes. A similar observation by Dautrebande and Haldane[76] with an increase in the bradycardia as the pressure was raised to 2 atmospheres was explained by these authors as a possible protective mechanism against excessive changes in respiratory metabolism. Bean found a definite decrease in pulse rate in dogs subjected to 4 to 5 atmospheres of oxygen[17,18] which he compared to a similar result from the administration of carbon dioxide. Richards and Barach[143] kept 2 normal subjects in 0.5 atmosphere of oxygen for 7 days, and while there was no appreciable change in cardiac output, they noticed a fall in pulse rate. Shaw, Behnke and Messer[151] concluded from their experiments on dogs that the first sign of oxygen poisoning was a fall in blood pressure, a point which was disputed by Bean and Rottschafer[25] who found that the blood pressure change was not consistent in dogs at 5 or 6 atmospheres. They also found that the bradycardia was dependent upon an intact vagus, a point later substantiated by Bean and Whitehorn.[26] On the other hand, when observing men in 3 and 4 atmospheres of oxygen, Behnke, Forbes and Motley[33] noticed that a rise in blood pressure accompanied the other signs of poisoning, which included pallor and an increase in pulse rate. Becker-Freysang and Clamann,[27] after 24 hours in 0.9 atmosphere of oxygen, found no marked change in their pulses, with the exception of an attack of paroxysmal tachycardia (which is not necessarily related to the oxygen); they made no measurements of blood pressure. Schwab, Fine and Mixter[148] observed a bradycardia of 60 for the first $\frac{1}{2}$ to 1 hour in patients breathing 1 atmosphere of oxygen; there was no change in blood pressure or color in 3 hours. However, at 7 hours in 1 atmosphere, Behnke[28] found that healthy males showed a rise in pulse rate accompanied by pallor, which was replaced by flushing as the time was extended. This was explained as a possible sympathetic stimulation followed by parasympathetic, suggesting the action of a histamine-like substance.

In connection with this, Willmon and Behnke[170] found 1 man who when exposed repeatedly to 2.5 atmospheres of oxygen, developed an allergic response, characterized by dermatitis and generalized wheals which were relieved by a preparation of histaminase. However, no similar cases have been found in the literature.

Several less clearly defined symptoms of oxygen poisoning are described in man and animals. Malaise is described by Becker-Freysang and Clamann,[27] perhaps due to the concomitant development of pneumonia; anorexia is described by Binger, Faulkner and Moore,[42] Boycott and Oakley,[49] and Paine, Lynn and Keys;[137] nausea and vomiting were observed by Becker-Freysang and Clamann[27] and Behnke[28] who also found several subjects under oxygen at 1 atmosphere who complained of substernal distress.

Summarizing the cardiovascular changes, although there is not complete unanimity, the bulk of evidence shows that high tensions of oxygen are usually accompanied by a bradycardia which is apparently of vagal origin. As to blood pressure variations, there is neither sufficient agreement nor evidence to warrant a conclusion. Similarly, the observations are too few and scattered to allow a generalization about other symptoms, which appear to vary considerably.

Pathologic Anatomy. Since a review of the symptomatology seems to warrant the conclusion that at tensions of oxygen up to 2 atmospheres, the damage is chiefly pulmonary, and at higher pressures it is chiefly upon the central nervous system, one looks first for lesions in these 2 systems.

Noting that oxygen at a greater concentration than 0.6 atmosphere is toxic, Armstrong[11] stated, "The pathology in these cases indicates that the toxic action is restricted to the respiratory tract with congestion, edema, pneumonia and death occurring in that order." Behnke[28] stated that pure oxygen is a pulmonary irritant. Shilling and Adams[152] concluded, "the convulsions and pulmonary damage are separate, unrelated phenomena, both caused by the high tension of oxygen but acting in different manners." Unfortunately, there have been no exhaustive studies made of nervous system lesions following oxygen poisoning, and there is no evidence that any occurs. In fact, with the exception of non-specific changes in a few other organs to be mentioned later, pathologic changes appear to be limited to the lungs. Hill[95] credits L. Smith with the discovery that a slow increase of oxygen pressure causes congestion of the lungs, with the result that there is no "quick rise in oxygen tension in the blood, and so the convulsions fail to appear." Hill describes edema and exudate as the chief features of lung pathology. The first detailed studies, however, were made by Karsner[112] and by Karsner and Ash[113] of both gross and microscopic changes following exposure to oxygen at 0.8 to 1 atmosphere. The lungs of rabbits so treated showed congestion, edema, epithelial degeneration and desquamation, fibrin formation and finally pneumonia, as the time of exposure increased to 48 hours, and as the concentration of oxygen was raised from 0.5 to 1 atmosphere. Smith, Bennett, Heim, Thomson and Drinker[157] carried out a similar study with rats exposed to 4 atmospheres of air pressure (equivalent to 0.8 atmosphere of oxygen). They found progressive cellular hypertrophy and hyperplasia of the alveoli which persisted for months after return to normal pressure. These changes occurred in both young and old rats, but to a greater extent in the older ones. The final histologic structure resembled an exaggeration of that normally found in unexposed young rats. The younger animals and those that had been previously exposed showed an increased tolerance or adaptation to high pressures. The possible protective function of these structural changes was suggested by further investigation along the same lines by Smith, Heim, Thomson and Drinker,[158] who also found thickening and hyalinization of the walls of pulmonary arterioles and large arteries, with occasional thrombosis. The vessels were said to resemble those seen in chronic nephritis. Hederer and Andre[91] also found greater tolerance to oxygen in young than in older rabbits; however, rather than an increased tolerance with previous exposure, they found increased sensitivity. A similar sensitization was found by de Almeida.[78] Behnke *et al.*[36] demonstrated pulmonary pathology in part of a series of dogs exposed to 4 atmospheres of oxygen, but found the onset of convulsions the same as in those without such changes. The basic pulmonary changes consisting of edema, exudation and congestion were all reported in various species by Bornstein and Stroink,[48] Achard, Binet and Leblanc,[1] Achard, Leblanc and Binet,[2] Binger, Faulkner and Moore,[42] Pflesser,[140] Armstrong,[11] Orzechowski and Holzknecht,[135] Binet, Bochet and Bryskier,[41] Paine, Keys and Lynn,[136] and Paine, Lynn and Keys.[137] In addition, true hemorrhagic extravasation was observed in turtles exposed to 0.9 atmosphere of oxygen by Faulkner and Binger.[82] Other pulmonary signs of oxygen poisoning include massive pleural effusion, seen in rats exposed to 1 atmosphere by Boycott and Oakley,[49] pulmonary hemorrhage in several mammalians, seen by Shilling and Adams[152] in pressures as high as 4 atmospheres of oxygen, and atelectasis at 4 atmospheres, found in dogs by Behnke *et al.*[36] The literature contains no reports of pathologic studies

of the lungs of man dying from the effects of high oxygen pressure, but there is some indirect evidence. When Becker-Freysang and Clamann[27] exposed themselves to 0.9 atmosphere of oxygen for 60 hours, one of them developed all the signs of bronchopneumonia at the end of the experiment, and required 10 days to recover. Jenkinson[109] drew upon his experience in submarine salvage work to state that high concentrations of oxygen at atmospheric pressure administered for several hours may bring on consolidation.

There is no evidence in the literature of lesions in the central nervous system.

There is a scattering of observations on the lesions in other organs following exposure to high oxygen tensions. Achard, Leblanc and Binet[2] described congestion in abdominal viscera, de Almeida[78] found atrophy of the testicles in rats, and Paine *et al.*[136,137] observed intense contraction of the spleen and distention of the stomach.

In conclusion, with the exception of minor effects upon abdominal viscera, the morphological changes caused by high pressures of oxygen are apparently limited to the lungs and pulmonary vessels.

Blood. 1. *Formed Elements and Hemoglobin.* Surprisingly, the first record found in the literature of a quantitative report on hemoglobin and red cell changes in the circulating blood under the influence of increased oxygen tensions was that of Campbell in 1926,[59] who observed a decrease of both in rabbits breathing 0.5 atmosphere of oxygen for 5 weeks; this change he considered a part of acclimatization. In contrast, Achard, Leblanc and Binet[2] found, in short exposures (0.8 atmosphere for 2 days), a marked increase of both red and white cells in rabbits and guinea pigs. In later papers, Campbell[66] reports a decrease in hemoglobin and red cells in several species of mammals in oxygen at about 1 atmosphere, and in rats at 6 atmospheres.[57] Izumiyami[108] likewise found a diminution in hemoglobin and red cells in 7 human subjects breathing 1 atmosphere of oxygen.

Boycott and Oakley,[49] in order to determine marrow activity, estimated the reticulocytes as well as the hemoglobin concentration in rats in 0.5 and 0.65 atmosphere of oxygen for 2 months. While there was some diminution in the hemoglobin concentration at 65% atmosphere, they concluded from the reticulocyte counts that, if anything, marrow activity was merely slowed down. Anthony,[9] and Anthony and Beudenkopf[10] made extensive measurements of human red cells and hemoglobin while the subjects were breathing oxygen through masks for 20 minutes and found an average drop in number of red cells of 7.8% and in hemoglobin of 3.3% with a corresponding increase in the color index. While breathing 0.9 atmosphere of oxygen, Becker-Freysang and Clamann[27] found a slight increase in red cells, and Paine, Keys and Lynn[136] showed a rise in hemoglobin concentration in dogs breathing 1 atmosphere. Similarly, Binet, Bochet and Bryskier[41] found, after an initial fall, a marked rise in red cells of guinea pigs, mice and pigeons in 0.7 to 1 atmosphere of oxygen.

The possibility that high pressures of oxygen alter blood hemoglobin apparently has not been investigated. Brooks,[52] however, reports a study on the relation of the rate of oxidation of hemoglobin and the partial pressure of oxygen. In the presence of certain oxidizing agents the rate is maximum to an oxygen pressure of 22 mm. The significance of this to oxygen poisoning is unknown.

Summarizing the changes in blood cells and hemoglobin, while the majority of observers with the most extensive data report a decrease in

red cells and hemoglobin, a minority report the opposite. Symptoms of pulmonary irritation or variations in the concentrations of oxygen studied do not give a consistent explanation for the difference. There are only 3 reports in the literature of determinations of white cells. Achard, Leblanc and Binet[2] found them to be increased in guinea pigs and rabbits breathing 0.8 atmosphere of oxygen, and 1 of 2 human subjects studied by Becker-Freysang and Clamann[27] showed slight leukocytosis in 0.9 atmosphere. Behnke, Johnson, Poppen and Motley[34] found a slight leukocytosis without change in the differential from 2 hours exposure to 3 atmospheres of oxygen.

2. *Blood Chemistry.* Shilling, Thomson, Behnke, Shaw and Messer[153] determined a great many chemical blood and urinary constituents in animals exposed to high oxygen pressure, but found appreciable changes only in inorganic phosphate and sugar in the blood, which decreased. The drop in blood sugar sonfirmed a similar finding by Izumiyami[108] in man and animals. They also found a decrease in chlorides, serum albumin and viscosity, which returned rapidly to normal after removal from the oxygen. Although Shilling *et al.*[153] found no great change in non-protein nitrogen, Binet, Bochet and Bryskier[41] found a rise in blood urea and uric acid in mice, guinea pigs and pigeons, and Paine, Lynn and Keys[137] found a 6 mg. per 100 cc. rise in non-protein nitrogen in dogs. Campbell[63] reports a significant increase in blood histamine in rats following exposure to 5 atmospheres of oxygen (0.5 to 1.3 gamma per cc.), the meaning of which is not apparent since he also found that injection of histamine had no effect on oxygen poisoning. This may be related, however, to Willmon and Behnke's finding[170] of an allergic response to oxygen in a man, relieved by histaminase (see section on Symptoms).

The finding of an increase in blood lactic acid by Bean and Haldi[24] in dogs subjected to oxygen pressures up to 5 atmospheres is a solitary observation of uncertain meaning.

Summarizing the changes in the blood, high oxygen pressures seem to produce a small but significant decrease in red cells and hemoglobin, and a slight leukocytosis. Various minor changes in blood chemistry occur, including an increase in histamine, but the significance of these is not clear.

The Circulation and Peripheral Vessels. Little work has been done on the actual measurement of cardiac output and blood flow, and the few observations recorded are limited to the vessels of the brain and retina. Pulse rate and blood pressure apparently tend to be lowered, although there is little consistency in the literature (see the section on Symptoms). Hill[95] confirmed a number of early authors that in compressed air, the circulation is unchanged. The only recent observation on cardiac output is that of Richards and Barach,[143] who could find no change in normal subjects breathing 0.5 atmosphere of oxygen for 1 week; this concentration, however, is usually considered outside the toxic range.

Tinel[165] found that high oxygen caused a constriction of the vessels of the brain. Wolff and Lennox[172] inserted a window over the pial artery of cats and observed a slight decrease in its diameter with 1 atmosphere of oxygen. While breathing a mixture of 90% oxygen and 10% carbon dioxide, the vessels were dilated and the animals showed only the symptoms of carbon dioxide poisoning. When the blood bicarbonate was artificially increased 39% the vessels were constricted, and when the carbon dioxide content was lowered by the intravenous injection of lactic acid, there was dilatation. Cobb and Fremont-Smith[74] studied the retinal circulation in man and found a change in size and color of the veins, but under a mixture

of 90% oxygen and 10% carbon dioxide, so their results cannot be interpreted as due predominantly to the oxygen. Cusick, Benson and Boothby[75] also studied retinal vessels. They found that 30 minutes exposure to 1 atmosphere of oxygen caused a 10 to 38% decrease in the size of the vessels, the greatest change occurring in the veins. Finally, Bean[17,18] directly measured blood flow to the brain through an external cannula in dogs subjected to 4 and 5 atmospheres of oxygen, but could find no change.

The only conclusion that can be drawn from these data is that there appears to be a change in vascular diameter under the influence of high oxygen, but the extent of this change elsewhere than in the brain and its mechanism is not clear.

Metabolism. Measurements of the effect of oxygen pressures up to 1 atmosphere upon the total metabolism are naturally discussed separately from those made at higher pressures. The evidence available indicates that up to 1 atmosphere for relatively short periods oxygen has little or no effect. For example, Hill and Macleod[99] confirming the early work of Bert[40] found that 24 hour exposures to 1 atmosphere of oxygen slightly lowered the CO_2 output of mice. Benedict and Higgins,[39] measuring both CO_2 output and oxygen consumption reported 292 observations of the total metabolism on 6 normal men. They concluded from this abundant evidence that in men in the basal state "breathing 40%, 60%, and 90% oxygen . . . . there is no apparent difference between the metabolism as indicated by the gaseous exchange and the metabolism when breathing ordinary air."

As might be expected owing to additional physical solution, the initial uptake of oxygen by the intact animal rises with increased oxygen pressure. Hence the determination of metabolic oxygen is difficult until a steady state is reached. For this reason some observers have determined total metabolism by measurement of the CO_2 output only. However, Behnke, Johnson, Poppen and Motley,[34] as well as Benedict and Higgins[39] have excluded this factor of physical solution. For example, the former authors found in the case of 4 men breathing 1 atmosphere of oxygen that the oxygen consumption was constant for periods up to 4 hours, at levels not significantly different from the basal uptake as calculated from normal standards. But they did find an increased uptake during the first 20 minutes. They excluded increased physical solution and equilibrium with intestinal gases as the cause, but offered no further explanation.

No systematic studies on metabolism after prolonged (more than 24 hours) treatment with 1 atmosphere of oxygen appear to have been done. Since toxic symptoms usually supervene under these circumstances, changes should be expected. Stadie, Riggs and Haugaard[161] found that the respiration of slices of lung from dogs exposed to 1 atmosphere of oxygen for 48 hours was decreased by 30% from the controls. Loss of weight has been reported by Binger, Faulkner, and Moore,[42] and Smith, Heim, Thomson, and Drinker.[158] However, the usual concomitant anorexia may have been as much responsible as metabolic changes.

Summarizing the metabolic changes, one may conclude that up to 1 atmosphere the inhalation of oxygen for 24 hours produces no significant change in the total metabolism. From the time of the discovery of oxygen by Lavoisier many systems of therapy have been built upon the assumption that the metabolism in normal and diseased states can be altered for the better by the inhalation of high concentrations of oxygen. Aside from the one rational use, *i. e.*, the alleviation of oxygen unsaturation of the blood in pulmonary and cardiac conditions, such conceptions still remain in the

realm of fancy. However, the possibility that lactic acid accumulation, oxygen debt, and so forth can be altered during short periods of vigorous exercise is real and has been discussed by Clark-Kennedy and Owen,[72] and Hill, Long and Lupton.[94]

When the oxygen pressure is raised appreciably above 1 atmosphere the situation is very different. Bert[40] did numerous experiments on rats, mice, sparrows and dogs using both air and oxygen to give partial pressures of oxygen ranging from 1 to 5 atmospheres. "In summary," he stated, "consumption of oxygen, production of carbonic acid and urea, breaking down of glucose in the blood, all chemical phenomena which can be measured easily, appear to be considerably slowed down by the action of oxygen under high tension. And as these are the phenomena which determine the production of heat, it is not surprising to see that the temperature of the animals drops considerably. Nor is it astonishing to see that death is the consequence of such depression in the intensity of the physico-chemical acts of nutrition." (Hitchcocks' translation.) By inference, Bert concluded that the action of oxygen in decreasing metabolic processes is due to its inhibitory effect upon the oxidative enzymes of the tissues (*v. infra*). Hill and Macleod[99] measuring the CO_2 output of mice, rats, and young rabbits, found a decrease at air pressures above 5 atmospheres and at oxygen pressures above 1 atmosphere. This, they concluded, is a constant concomitant of oxygen poisoning. Bean[17,18] also found a definite decrease of oxygen uptake in dogs under 5 atmospheres of oxygen.

Blood Gas Equilibrium. *Oxygen.* The relation between inspired air and blood gases is affected by variations in respiratory movement caused by central nervous system response to toxic levels of oxygen (convulsions), and by possible morphological changes in the alveoli. Hence interpretations of gas equilibria within the organism should be cautious. Nevertheless, Behnke *et al.*[36] have clearly demonstrated a prompt equilibrium between alveolar and pulmonary blood oxygen in dogs under 4 atmospheres of oxygen.

Carbon Dioxide. In the problem of blood and tissue carbon dioxide under high oxygen, the Reviewers believe that there has been in the literature an unfortunate and erroneous use of the word "retention." They define retention as an *accumulation* of carbon dioxide in blood or tissues owing either to impaired transportation or faulty elimination. They distinguish from this an increase in the partial pressure of *free* carbon dioxide, which does not necessarily mean retention as will be pointed out later. They further distinguish retention from *diminished metabolic formation.* Without these distinctions, discussion of the literature becomes confusing.

The question of carbon dioxide output under high oxygen must be discussed in the light of: (1) possible associated change of total metabolism, or (2) true carbon dioxide retention. While there are many experiments showing that carbon dioxide output is diminished under moderate oxygen pressures (0.8 to 2 atmospheres), concomitant studies on oxygen uptake have as a rule not been made, hence decrease in metabolism cannot be eliminated as the cause. Such experiments were reported by Bert,[40] Achard, Binet and Leblanc,[1] Achard, Leblanc and Binet,[2] and Becker-Freysang and Clamann.[27] On the other hand, Hill and his co-workers,[95] in experiments at these relatively low pressures, while finding similarly diminished carbon dioxide output, also noted an associated fall in body temperature sufficient to indicate a markedly lowered metabolism. In other words, there is no evidence of a true carbon dioxide retention under

these conditions; rather, decreased metabolism is a sufficient explanation. However, Hill suggested pulmonary damage as the cause. While this might easily explain an increase in the partial pressure of blood or tissue carbon dioxide as observed in other types of pulmonary pathology, it could only explain a temporary and not a persistent diminution of carbon dioxide elimination and even less, the increasing diminution which Hill observed. Furthermore, there is no evidence that true retention of carbon dioxide as we have defined it occurs even under pressures of oxygen greater than 2 atmospheres. In fact, Behnke *et al.*[36] demonstrated in dogs that exposure to 3.8 atmospheres does not significantly alter the *arterial* P_{CO_2}, in sharp contrast to the increased P_{CO_2} of the *venous* blood generally observed. In the Reviewers' opinion this is proof that there is no interference with carbon dioxide transportation or elimination. Finally, Behnke and Stephenson,[37] quoting their own unpublished experiments, were "unable to demonstrate any retardation in the elimination of carbon dioxide from the tissues" under high pressures of oxygen.

On the other hand, there is clear evidence that the *partial pressure* of carbon dioxide in the tissues is *increased* by high oxygen. Hill,[96] Gesell,[88] Campbell[59–61] and Behnke *et al.*[36] all found an elevation of P_{CO_2} in tissues and *venous* blood. A consideration of the full evidence outlined above, particularly that of Behnke, has brought the Reviewers to the conclusion that this increased CO_2 pressure is not due to retention as they have defined it. In their opinion the interesting and unique change in the acid-base balance of the blood known as the loss of the dual function of hemoglobin, first outlined by Gesell,[88] is a sufficient explanation. This is discussed more fully later.

Summarizing data on blood gas equilibrium, while there is evidence that high oxygen pressure produces a decrease in oxygen uptake, and *pari passu* in carbon dioxide output, these changes can be fully explained on the basis of lowered general metabolism. Changes in the partial pressure of carbon dioxide constitute a separate problem, accountable for, we believe, by Gesell's hypothesis.

Influencing Factors. *Gases.* At the outset, the distinction must be clearly drawn between the specific effects of high pressures of gases other than oxygen, and the effects which such gases produce on the toxicity of oxygen. In the first case, for example, the well-known narcotic effect of nitrogen at high pressures of air characterized by stupor, mental torpor, and so forth might be so great *per se* as to obscure more or less the effects of oxygen. Thus, Behnke, Thomson and Motley[38] contrasted the above-mentioned symptoms of narcosis produced by 3 to 10 atmospheres of air pressure with the convulsions, syncope, and so forth described by Behnke *et al.*[34] as accompanying 4 atmospheres of oxygen. They infer that under air pressure, the symptoms are largely those of nitrogen narcosis. They were confirmed in this conclusion by the experiments of Case and Haldane.[69] No experiments have been reported which would suggest any influence of nitrogen upon the toxicity of oxygen. These specific effects of gases other than oxygen do not fall within the scope of this review, but are mentioned in order to make the distinction clear.

Possible toxic action of contaminants of cylinder oxygen as causal agents has been excluded by the early work of Bert,[40] who found symptoms with cylinder oxygen or compressed air to be solely a function of the partial pressure of the oxygen. Binger, Faulkner and Moore,[42] in studies on rabbits, also excluded ozone as a possible cause of poisoning.

The most important gas to be considered in conjunction with oxygen is

carbon dioxide, because it figures largely in Gesell's discussion of the "dual function of hemoglobin" (*q.v.*). Gesell's hypothesis states that oxygen at high tensions increases the sensitivity of the animal to the administration of carbon dioxide owing to the broken coördination of the dual function of hemoglobin. This leads to acidosis which is further augmented by the administration of carbon dioxide, as he found to be the case in experiments on rats. The opposite explanation is offered by the observations of Shaw, Behnke and Messer[151] who produced subnormal levels of alveolar carbon dioxide tension by artificial hyperventilation, and supernormal levels by increasing the carbon dioxide in the mixture breathed, in dogs subjected to from 1 to 4 atmospheres of oxygen. Although the signs of oxygen poisoning appeared with subnormal alveolar carbon dioxide, they were hastened by an elevated carbon dioxide. The authors found, like Gesell, that elevated tensions of carbon dioxide which were not toxic at 1 atmosphere became associated with toxic symptoms in the presence of 4 atmospheres of oxygen; but, unlike Gesell, concluded that carbon dioxide might increase the toxicity of the oxygen or the sensitivity of the tissues to oxygen. However, it is obvious that no choice can be made between the two explanations on the basis of these experiments. In any case, since toxic symptoms of oxygen were found at subnormal levels of alveolar carbon dioxide, Shaw, Behnke and Messer concluded that carbon dioxide plays only a secondary rôle in oxygen poisoning. (For further discussion, see the sections on the Dual Function of Hemoglobin and Metabolism.)

In a more restricted experiment, discussed elsewhere with the question of circulation, Wolff and Lennox[172] found very slight arterial contraction under the influence of pure oxygen, and distinct dilatation when 10% carbon dioxide was added. In this respect, then, carbon dioxide appears to exert an influence exactly opposite to that of oxygen.

Hill[96] exposed monkeys, guinea pigs, rats, and goats to a mixture of 5% carbon dioxide in 1 atmosphere of oxygen, and then oxygen up to 5 atmospheres. The result was that the critical pressure of oxygen needed to produce convulsions within a given time, and the time necessary to produce them at a given oxygen pressure, were both distinctly lowered. He concluded that an "increase in carbon dioxide tension in the tissues is a factor in the production of convulsions which follow exposure to high pressures of oxygen." The same result was achieved by Massart[127] on mice, using 5% carbon dioxide in oxygen at 4 atmospheres, but his explanation is that respirations are increased (by the carbon dioxide) and hence there is a more rapid arrival at equilibrium with the oxygen. This explanation is untenable in view of the facts that respiratory rate is not materially affected by high air pressures (see the section on Symptoms), and that alveolar and blood oxygen are in equilibrium when pure oxygen is breathed (Behnke *et al.*[36]). The acceleration of oxygen toxicity by admixed carbon dioxide was also observed by Hederer and Andre.[91]

Libbrecht and Massart,[121] in an unconfirmed single report, stated that hydrogen had an antagonistic action to that of oxygen at high pressures. Mice, which had severe convulsions at 4 atmospheres of pure oxygen, when subjected to the same oxygen pressure plus 6 atmospheres of hydrogen, developed no convulsion but severe pulmonary symptoms only, although in the latter case the animals died sooner. This would indicate that the hydrogen acts as antagonist only to the effects of oxygen upon the nervous system as distinguished from those upon the pulmonary system.

Other Factors. Paul Bert[40] first observed, in conjunction with the general slowing of physiologic processes under compressed air, that body tempera-

ture was lowered. Hill[95] confirmed this, and made the further observation, which is difficult to explain in the light of later experiments, that he could protect animals to some extent by warming the pressure chamber. The first suggestion of the opposite effect of external temperature was given by Faulkner and Binger[82] who found that while turtles were more resistant than warm-blooded animals to 0.9 atmosphere of oxygen ordinarily, they became fully as susceptible to lung damage when the temperature was raised to 37.5° C. Perhaps this is owing to the increased metabolism of poikilothermous animals at elevated temperatures, a well-known phenomenon. Later, Campbell[62–64] made quantitative studies of the temperature effect on rats and found that a decrease in body temperature of about 10° C. increased the survival factor by about 10 times at pressures up to 6 atmospheres. The only temperature studies on men that have been done were concerned with the effects of carbon dioxide and nitrogen in compressed air, by Case and Haldane.[69]

One would expect that muscular exercise would alter the effects of high oxygen pressures, but no thorough investigations have been directed at this question. However, Bornstein and Stroink,[48] while finding no symptoms in themselves at rest in 45 minutes at 2 atmospheres, brought on cramps, clonic muscular spasms and hyperactivity of deep reflexes by exercises. Hill, Long and Lupton[94] studied the effect of exercise in man, but only at the non-toxic level of 0.5 atmosphere; they found a 10 to 50% increase in oxygen uptake, which was not due to increased saturation of the blood. It would be valuable to extend this investigation to higher concentrations of oxygen.

A number of drugs have been found to affect oxygen poisoning. The first investigation of drugs by Campbell[59] disclosed no effect by thyroid medication, although in later works,[63,65,66] he found that thyroxin and to a lesser degree certain other drugs, definitely enhanced the effect of oxygen on rats. In substantiation, he also found that the survival rate was increased following either thyroidectomy or hypophysectomy (to remove the thyrotropic effect of the posterior pituitary). He found that histamine had no effect (see section on Circulating Blood), but that oxygen poisoning was increased by dinitrophenol, ac-tetrahydro-beta-naphthylamine, adrenalin, extract of the posterior lobe of the pituitary, insulin, eserine and atropine. Marshall and Rosenfeld[126] found that oxygen increased the depression of respiration caused by anesthetics (including barbiturates and morphine, but not chlorbutanol, urethane, paraldehyde or alcohol) apparently by removing otherwise effective anoxemic stimuli which act through the sino-aortic mechanism. On the other hand, addition of carbon dioxide to the oxygen failed to prevent this enhanced depression, which suggests an effect due to a specific action of oxygen. A conflicting observation was made by Hederer and Andre[91] that barbiturates retarded the convulsions produced by oxygen, but as might be expected, strychnine enhanced them.

Finally, Campbell[62] and de Almeida[78,79] both found a marked increase in resistance to oxygen in rats that had been starved, and Smith *et al.*[158] found that the tolerance of rats was decreased late in pregnancy.

In summary, there are several factors which influence oxygen poisoning. Carbon dioxide clearly exerts an influence, apparently secondary, either by enhancing the effect of oxygen, or by contributing an independent effect enhanced by the oxygen. There is some evidence that it counteracts peripheral vascular contraction by oxygen but to what extent the latter occurs is not clear. Hydrogen appears to retard the effects of high

oxygen pressures, but there is no evidence as to an effect by nitrogen. Oxygen poisoning has been well shown to be diminished by a lowered body temperature, and *vice versa*, as well as by starvation, but to be augmented by several drugs, notably thyroxin and certain respiratory depressants.

Tolerance, Adaptation and Oxygen Therapy. Any discussion of oxygen poisoning naturally requires consideration of the maximum non-deleterious dose of oxygen at various pressures above the normal of 0.2 atmosphere. The observations in the case of man are more complete and consistent than those for other species, and will be considered first because of their importance.

The lowest concentration of oxygen at which physiologic changes have been noticed was reported as 45% by Richards and Barach,[143] who found that 2 normal subjects kept at this level for a week showed a fall in pulse rate and a slight rise in total carbon dioxide content of the blood. There were no changes in respiratory metabolism, cardiac output or excretion of electrolytes and water. Patients with cardiac insufficiency, however, showed good therapeutic response to oxygen at 0.4 to 0.5 atmosphere for periods up to 7 months without demonstrable signs of oxygen poisoning.[144] Binet *et al.*[41] described some minor physiologic changes in rabbits at 0.6 atmosphere. With these exceptions, the maximum oxygen concentration that can be inhaled by man and other warm-blooded species indefinitely without harm was given as approximately 0.6 atmosphere by Barach,[14] Boycott and Oakley,[50] Binet *et al.*,[41] Behnke and Shaw,[35] Becker-Freysang and Clamann[27] and Trusler and Meiks.[166]

Recently developed methods have made possible the use of oxygen at nearly 1 atmosphere for certain purposes, as described for example by Boothby[46] and Boothby, Mayo and Lovelace.[47] Behnke *et al.*[34] described symptoms occurring in 0.96 atmosphere oxygen at various times up to about 4 hours, which they gave as the limit of safe exposure. The important observation of Marshall and Rosenfeld[126] was discussed above, that under certain conditions of respiratory depression by anesthetics, the administration of oxygen will further depress respiration. While Anthony[9] and Anthony and Beudenkopf[10] describe a fall in the number of blood cells and hemoglobin produced by inhalation of approximately 1 atmosphere of oxygen for periods as short as 15 minutes, it is doubtful whether this can be considered a toxic effect, since it is reversible and was not reported as accompanied by symptoms. Fine, Hermanson and Frehling[83] reported no toxic symptoms from the use of 0.95 atmosphere of oxygen when the patients were taken out of the tents at least ½ hour out of every 4 to 8 hours. Schwartz and Malikiosis[149] reported the sudden development of clonic spasms and collapse in subjects who were given 1 atmosphere of oxygen following a period of breathing air at diminished pressure. Boothby[46] concluded that oxygen at 1 atmosphere may be administered for 24 hours, and Boothby, Mayo and Lovelace[47] reported the administration of 1 atmosphere of oxygen to 800 patients with no evidence of the development of pulmonary irritation in less than 48 hours, and none in several days with intermittent administration. Whether in all cases these patients were inhaling 1 atmosphere of oxygen is uncertain since the report gives no analyses of circumambient oxygen. Furthermore, the important factor of intermittence for feeding, medication, and so forth was not included in the data. The authors recommend, in conclusion, that 1 atmosphere of oxygen may be given for 36 to 40 hours, but then must be reduced to 0.6 atmosphere. Becker-Freysang and Clamann[27] felt no symptoms for 24 hours in 0.9 atmosphere of oxygen. Behnke,[28] on the other hand,

found that healthy males show a slowing of the pulse rate and pallor in 7 hours at 1 atmosphere, and beyond this time, an increase in respiratory depth with nausea and flushing. In a later paper, Behnke[30] stated that 1 atmosphere can in most cases be inhaled for 17 hours without injury, although some subjects have complained of substernal distress in 7 hours, and 1 subject developed an allergic response. Moody and Howard,[132] using an oxygen tent, observed, as previously mentioned, convulsions in a child, who spent 6 days intermittently in an atmosphere which, although not measured, was assumed to be in excess of 0.7 atmosphere of oxygen. The child, 2 years old, had pneumonia and had given a good response to sulfonamide therapy with the exception that cyanosis had persisted. The convulsions were relieved by removal from the tent, recurred on re-entrance, and were again relieved on removal. For this reason, and because of the absence of any other abnormal findings including fever, they were attributed to the oxygen.

The conservative conclusion by the great majority of those experienced in oxygen therapy is that 0.6 atmosphere of oxygen is relatively safe for an indefinite period, and 1 atmosphere for about 24 hours, with due consideration for the factors discussed elsewhere. Ruff and Strughold, quoted by Becker-Freysang and Clamann,[27] described what they called the "paradoxical oxygen effect." Subjects breathing oxygen at 1 atmosphere for a period of time, when transferred to air at somewhat diminished pressure, developed clonic spasms and collapse. This phenomenon has been observed in interceptor pilots who breathe oxygen at ground level for purposes of de-nitrogenation and then ascend quickly to high altitudes. It probably is not related to the problem of oxygen poisoning, but is perhaps explainable in the light of the observations of Watt, Dumke and Comroe,[168] discussed in the section on Respiratory Symptoms.

Young animals appear to be relatively resistant to the toxic action of high pressures of oxygen.[91,157] For example, Smith, Bennett, Heim, Thomson and Drinker[157] found that young rats were not so readily poisoned by exposures to 0.8 to 1 atmosphere of oxygen as were older ones, a fact which they were inclined to attribute to morphologic differences in pulmonary structure. This increased tolerance has also been observed in the human by Chapple.* Employing a special incubator[69a] which permitted treatment without intermittence at oxygen pressures never less than 0.85 atmosphere, he has treated premature infants weighing less than 3 lbs. for periods frequently as long as 3 weeks. During treatment the humidity is maintained close to 100%. Under these circumstances, he observed no poisonous action of oxygen whatever, and is convinced that this form of oxygen therapy is of great value in this type of case.

It has been suggested that part of the deleterious action of oxygen especially upon the lungs may be due to its dryness when administered. No papers discussing the possible relation of humidity to oxygen poisoning have been found.

The few conflicting reports concerning adaptation are mentioned in the discussion of morphology and circulating blood, and allow only the conclusion that if adaptation occurs, it is due to the protective action of pulmonary pathology, and possibly, as pointed out by Campbell,[60] to the decrease in hemoglobin and red cells (*v. supra*).

In connection with the use of oxygen at pressures about 1 atmosphere,

* Dr. Charles C. Chapple, of the Department of Pediatrics, University of Pennsylvania, has courteously permitted us to quote his work in advance of its publication in this Journal.

for example in preliminary de-nitrogenation of divers, Behnke and Shaw[35] have emphasized the importance of a thorough knowledge of tolerance levels. In view of the scattered nature and inconsistency of the observations at higher pressures, particularly in animals, the summary given in Table 1 of tolerance levels found by various investigators will serve as useful a purpose as an extended discussion of all the reports. As was pointed out earlier, the safe time limit can be seen to drop with increasing rapidity as the oxygen pressure is raised above 1 atmosphere. One explanation for this phenomenon, which supposes a protective effect at low pressures by pulmonary pathology, is discussed in the section on Pathology. It can also be seen by inspection of the table that there is a marked variation in tolerance to oxygen among different species. For example, while most mammalians have almost instantaneous convulsions at 5 atmospheres, Cleveland's observations[73] on cold-blooded vertebrates show a markedly greater tolerance of these species.

TABLE 1.—A COMPARISON OF THE LEVELS OF OXYGEN POISONOUS TO VARIOUS SPECIES, FROM AVERAGE FIGURES IN THE LITERATURE

Oxygen tension (atmospheres)	Animal species	Lethal time (hrs.)	Asymptomatic time limit for man	Reference numbers
0.45	.....	...	7 days	95, 143
0.6	.....	...	Indefinite	14, 27, 35, 41
0.7+	.....	...	6 days	49, 132, 166
0.8	Rabbit	192	...	14, 42
0.85 to 1	Premature infants	...	> 3 weeks	Chapple (personal communication)
0.89 to 0.9	Mouse	36	24 hours	27, 140
	Dog	120	...	136, 137
0.95 to 0.96	Rat	144	...	34, 57, 154
	Cat	72	...	136, 137, 140
	Dog	108		
0.99 to 1	Dog	48	7 to 40 hr.	28, 30, 34, 46, 47, 136, 137, 140
2.0	Mammalians	4	0.75 to 3 hr.	34, 48, 96
3.0	.....	...	0.50 to 2	34, 96, 109
3.5	Frogs	65	...	73
	Salamanders and goldfish	50 to 60	...	73
4.0	Mammalians	0.48	0.20 to 0.70	34, 96, 164
5.0	Mammalians	0 to 2 (rats)	...	48, 96
7.0	.....	...	0.10	90
8.0	Rats	0	...	48
9.0	.....	...	0.05	109
50.0	Rats, mice, etc.	0	...	99

One may conclude that the maximum non-deleterious dose of oxygen which can be used for indefinite periods appears to be 0.6 atmosphere, while for 1 atmosphere the time limit is 24 hours. Chapple's experience (*v. supra*) would appear to exclude premature infants from these limitations. The evidence is not sufficient to say that adaptation occurs to an important degree. Tolerance to high oxygen decreases rapidly with increasing pressures above 1 atmosphere, and is in general lowest for mammalians and greatest for cold-blooded species.

Oxygen Aero-embolism From Rapid Decompression. There is a sole report in the literature: Hill,[95] in experiments on toads, rats and guinea pigs, showed the formation of bubbles in the heart and other organs following rapid decompression from high pressures of pure oxygen, but the

pressures used were very high, namely, 15 to 20 atmospheres. It is probable that decompression from lower pressures (2 to 5 atmospheres) would not be accompanied by aero-embolism, because oxygen, unlike nitrogen, is rapidly used for metabolic purposes and therefore would most probably not persist, to form gas emboli.

Isolated Surviving Tissue. The experimental work in this category is limited. Bert (1878)[40] reported that the oxygen uptake and CO_2 output of strips of beef muscle, when subjected to high oxygen, was diminished compared to controls.

Most of our later knowledge of this phase of the subject is due to the excellent series of papers by Bean and his associates.. Bean and Bohr[23] found in the case of smooth muscle (duodenal and pyloric sphincter muscle of rabbits) subjected to 6 atmospheres of oxygen, a definite pattern of low peristalsis and spasm similar to that produced by HCN but different from that by atropine. He concluded that this was owing to a direct action of oxygen upon the enzymatic systems of the tissues resulting in low oxygen utilization, a condition which he called "hyperoxic anoxia." The effect was reversible only after comparatively short exposure to relatively low pressures; no reversibility occurred after exposure to high pressures. With radial beef iris muscle, Bean and Bohr[22] observed a decrease of tonus when the muscle was subjected to 4.8 atmospheres of oxygen. Controls in air at the same pressure were unaffected. Recovery of normal tone followed removal from the high pressure. Atropinization did not change the effect of high oxygen. The authors concluded that the pupillary dilatation observed at high pressures of oxygen is not necessarily dependent on central nervous or hematogenous connections. Studying mammalian smooth muscle (rabbit duodenal strips) Bohr and Bean[44] found a decrease of tonus after 2.5 hours at 6 atmospheres of oxygen. Reversal to normal occurred if the high oxygen period was relatively short, but otherwise a periodic tonic spasm supervened. They stated that the action is not due to an epinephrine-like substance at the myoneural junction. Bohr and Bean[43] studied the action of the isolated frog heart in 5 atmospheres of oxygen. They found an initial increase followed by a delayed slight decrease in the strength of the beat. Later there was a loss of automaticity, but irritability remained even after the beat had ceased. Recovery after decompression was observed if the experiment was terminated before the complete cessation of cardiac activity.

The same authors,[21] using isolated striated muscle of the frog, found after exposure for 1.5 to 5 hours to oxygen at 5 atmospheres an initial increase of contraction followed, however, by a marked decrease in response to electrical stimulation of muscle or nerve compared to controls in air at elevated pressures. Usually no recovery was observed after 2 exposures. Their data led them to believe that the effect is mainly if not all upon the muscle rather than the nerve fiber or the myoneural junction. Hill and Macleod[99] found that the thin sartorius muscle of the frog, after 1 hour exposure to 50 atmospheres of oxygen, showed a greatly diminished height of contraction and a prolonged latent period. The gastrocnemius muscle, however, appeared relatively little affected.

Bean and Bohr,[21] in discussing the problem of oxygen poisoning, laid considerable stress upon the loss of the dual function of hemoglobin as the main cause of tissue dysfunction. They attributed to the "accumulated carbon dioxide and acidity resulting from inadequate reduction of hemoglobin a more significant etiologic factor in the early signs and later events of oxygen poisoning" than the "direct" toxic action of high oxygen upon

the enzyme systems involved in metabolic processes. This aspect of the subject is discussed more fully later.

Hill and Macleod[99] observed that a frog's heart continued to beat after 1 hour's exposure to an oxygen pressure of 50 atmospheres. Cold-blooded animals are known to be relatively resistant to oxygen poisoning, nevertheless this is an extraordinarily high pressure of oxygen to be survived by tissues.

Burrows[54] found in the case of tissues from chick embryos that the rate of growth is slightly more rapid in an atmosphere of pure oxygen, but the total growth is no greater than at lower partial pressures. He did not study the effect of pressures greater than 1 atmosphere.

Summarizing the only evidence available on isolated surviving tissue, it shows a distinct decrease in the function of smooth and striated muscle, but no apparent effect on myoneural junctions. Other tissues have not yet been sufficiently studied to warrant a conclusion.

Neoplasia. A brief chapter on this subject has accumulated in the literature. Fischer and Andersen[84] reported that under increased oxygen pressure sarcoma cells were killed more quickly than normal tissue cells in artificial culture media. Their data, however, are not convincing. Later, Andersen and Demuth,[8] experimenting on 500 mice, reported that in some animals pre-treated intravenously with copper or selenium (having tumor affinity and possibly catalytic action) and kept in 1.5 to 2 atmospheres of oxygen for 24 hours, transplanted carcinomata could be made to disappear completely. Oxygen or the metal alone did not give the effect. Occasionally permanent recovery followed, but in most cases the tumor reappeared from incompletely necrotized tissue. Still later, Fischer and Andersen[85] again found with transplanted tumor in mice that treatment for 24 hours with 1 atmosphere of oxygen resulted in the disappearance of the tumor. Better results were obtained when the oxygen treatment was combined with copper or selenium injections. However, these results were obtained only if the treatment was given during the first week after the transplantation and they surmised that similar therapeutic success is doubtful in the case of humans. De Almeida,[79] using starved rats to increase oxygen resistance, found that sarcoma (fusiform cell type of Ruffo) could be made to disappear following treatment with oxygen at 1 to 8 atmospheres for 24 hours to 20 minutes. His best results were obtained with 6 atmospheres of oxygen for 2 hours. The tumors were completely destroyed by a single treatment; 10 days later they were red, impregnated with blood, and microscopically none of the tumor tissue was preserved. De Almeida expressed intentions of building a chamber for the treatment of human cases to be reported on later. We have been unable to find further reference to such work. Campbell[58] subjected de Almeida's work to close scrutiny. Using Bashford's mouse carcinoma (No. 63), Twort's mouse tumor, or Walker rat tumor, he was unable to demonstrate any effect upon the histology after treatment of the animals with 4 to 5 atmospheres of oxygen for 1 hour. Nor was any effect found on spontaneous mouse mammary carcinoma.

The possibility, then, that high oxygen pressures might differentially injure or kill malignant cells either in culture or *in situ* remains unsettled in view of these interesting but conflicting reports.

Plants and Microörganisms. Scattered observations indicate a toxic action of high oxygen pressure upon forms of life other than mammalian. Bean,[19,20] for example, observed that the growth of pneumococcus is a function of the oxygen pressure, being maximum at about 1.2 atmospheres

and then declining, to cease at a pressure of about 2 atmospheres. The organisms could be killed by exposures to 5.8 atmospheres for 1 to 2 hours.

Boothby[46] discussed the possibility of the bactericidal action of oxygen at 1 atmosphere in cases of gas gangrene and tetanus.

Karsner, Brittingham and Richardson[114] found that the growth of most of the common pathogenic bacteria is inhibited by 1 atmosphere of oxygen. The pneumococcus was an exception.

Novy and Soule,[134] studying the tubercle bacillus, observed excellent growth at 0.4 to 0.5 atmosphere of oxygen but reported definite inhibition at 0.8 to 1 atmosphere.

Rahn and Richardson[142] studied the growth of *Streptococcus lacticus*, *Pseudomonas fluorescens* and *Bacillus subtilis* under high oxygen pressures. The first was greatly inhibited by oxygen at 1 atmosphere. The others were uninfluenced.

Thaysen[163] reported that the growth of a number of common organisms was greatly retarded at 10 atmospheres of oxygen. The effect was dependent upon the temperature, being lethal if the temperature was raised slightly above the optimum for growth. This observation is similar to those on higher organisms, discussed elsewhere.

Massart[128] reported that the respiration of yeast was decreased by exposures to 3 to 5 atmospheres for short periods of time (15 to 60 minutes). He attributed this to a partially irreversible oxidation of cytochrome which in the oxidized state, he assumed, is without respiratory function. Albaum, Kaiser, and Eichel[6] showed that oat grains soaked for 24 to 48 hours in water saturated with oxygen at 1 atmosphere manifested inhibited growth. Albaum, Donelly and Korkes[5] found a similar effect with oat seedlings associated with a lowered catalase and endogenous dehydrogenase activity. Cleveland,[73] in an interesting paper, reported that intestinal protozoa infesting termites, earthworms, cockroaches, frogs, salamanders and goldfish, were killed *in situ* by 3.5 atmospheres of oxygen without injury to the hosts. However, intestinal trichomonas of rats or man were not killed *in vitro* by such pressures.

Summarizing the evidence on microörganisms, high oxygen causes toxic effects which are similarly enhanced by elevated temperature, upon them as upon vertebrates.

Dual Function of Hemoglobin. The expected increase of saturation of arterial and particularly venous blood resulting from high oxygen pressure was first observed by Bert[40] and confirmed in extensive studies on human subjects by Hill.[95] Increase of physically dissolved oxygen as well was demonstrated by Wolff and Lennox[172] in cats at about 1 atmosphere, and by Behnke *et al.*, whose experiments are fully discussed below.

One hypothesis explaining the toxic effect of excess oxygen in the intact mammalian organism resulting from these observations, and first proposed by Gesell,[88,89] postulates that part of the "dual function" of hemoglobin is lost owing to the fact that blood oxygen in physical solution is sufficient in whole or in part for metabolic needs; hence little or none is supplied by reduction of the hemoglobin. The mechanism by which this loss produces its effects is illustrated by Figure 1. This shows the relation between the total CO_2, the pH, and P_{CO_2} of the whole blood. The *arterial* point, *A*, is on the totally oxygenated line, and the blood (the subject breathing air) contains 50 vol. % of CO_2, the pH = 7.400 and the P_{CO_2} is 40 mm. Hg. (Assume for simplicity that the blood is 100% saturated and contains hemoglobin equivalent to 20 vols. % of oxygen.) The venous point is derived thus: in the normal subject at rest, the mean CO_2 content

of *venous* blood is 55 vols. %, approximately 5 vols. % greater than the CO_2 of *arterial* blood. If the respiratory quotient is average, *i. e.*, 0.8, there will be 5/0.8 = 6.2 vols. of oxygen consumed per 100 cc. of blood. The blood will then be 69% saturated, and the CO_2-pH line of the whole blood will be that indicated by the dashed line intermediate between the fully oxygenated and fully reduced lines. Hence the *venous* point will be

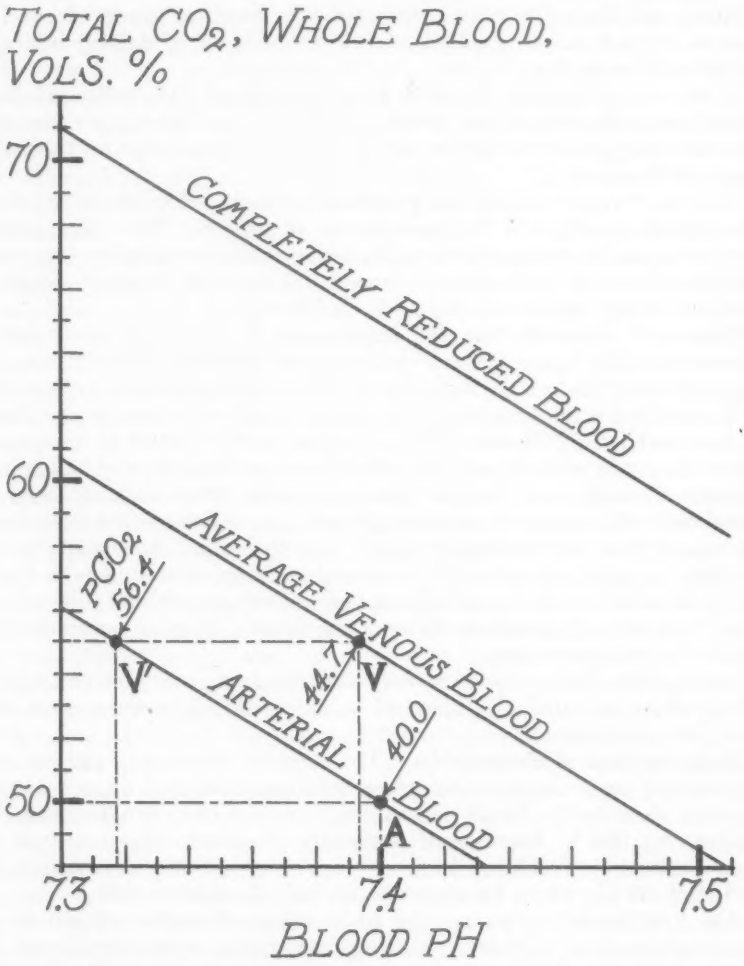

FIG. 1.—Schematic diagram illustrating the loss of the dual function of hemoglobin when sufficient oxygen (2.6 atmospheres) is inhaled to supply metabolic needs by physical solution.

at *V*. The pH and P_{CO_2} will then be 7.395 and 44.7 mm. Hg respectively. By reduction of the hemoglobin, 4.73 vols. % of CO_2 (95% of the total) are taken up without any change in pH, while only 0.27 vols. % (5% of the total) are taken up by the pure buffer action of the blood. The pH change is thus very small, *i. e.*, only 0.005 pH. The situation is quite different if the subject is breathing oxygen under increased pressure. For example, the solubility of oxygen in water is 2.4 vols. % per atmosphere

and hence (neglecting the diminished solubility in blood since the calculations are approximate and illustrative only) at 6.2/2.4 = 2.6 atmospheres oxygen pressure the oxygen in physical solution alone would be sufficient to take care of the metabolic needs. There would be no reduction of the hemoglobin whatever and the line showing the relation between total CO_2 and pH of the venous blood would be the fully oxygenated one. The venous point would then be at V' and the pH and the P_{CO_2} would be 7.317 and 56.4 mm. Hg respectively. The elimination, owing to the presence of sufficient physically dissolved oxygen for metabolic needs, of this important function of hemoglobin, namely, release of base upon reduction, increases the changes of the pH by 0.078 and of P_{CO_2} by 11.7 mm. Hg beyond those occurring normally. The increased metabolism of exercise does not affect the above conclusions. For it is easy to show, on physicochemical grounds only, that when the CO_2 output and oxygen uptake increase *pari passu* the calculated values of pH and P_{CO_2} of the venous blood are essentially those given for the basal state.

If this mechanism is the sole one operating when oxygen at high pressures (>2.6 atmospheres) is inhaled, we would expect to find a decrease below the normal venous pH of about 0.08, and an increased venous partial pressure of CO_2 of about 12 mm. Hg. Bean,[18] using the manganese dioxide electrode, was successful in measuring such changes of pH of blood in dogs breathing oxygen at elevated pressures, the venous blood being arterial in color. No P_{CO_2} values were reported. Bean, in agreement with Gesell, attributed considerable significance to these changes. Campbell has reported extensive observations on the partial pressure of CO_2 in the tissues of animals breathing air, and oxygen under high pressures. He injected bubbles of air or nitrogen into tissues or into the peritoneal cavity and after sufficient time had elapsed for equilibration with tissue CO_2, removed the gas for analysis. He found quite consistently, compared to normal conditions, that under high oxygen tensions the partial pressure of the CO_2 in tissue was elevated by 15 to 20%. This led him to conclude that a major effect of oxygen poisoning is a "retention" of free carbon dioxide by the tissues. Paine, Lynn and Keys,[137] on the other hand, studying the gases in artificially obstructed intestinal loops of dogs in 1 atmosphere of oxygen, found no changes in the carbon dioxide levels from those found in similar animals breathing air, although the oxygen tensions rose as was anticipated. These observations are suggestive, but the critical experiment to test Gesell's hypothesis requires determination by the glass electrode of the actual pH, the analysis of the arterial and venous blood for total CO_2, and the calculation from these data of the P_{CO_2} of the venous and arterial blood under conditions of high oxygen pressure.

Behnke *et al.*[36] have approached this ideal experiment so closely that their observations may be accepted with confidence. Arterial and venous blood from dogs under high oxygen pressures was analyzed for total CO_2. Samples of the same blood were then equilibrated with known pressures of CO_2 and the total CO_2 content determined. From these data the authors constructed log CO_2—log P_{CO_2} lines according to the method of Peters,[139] and by interpolation determined the P_{CO_2} of the original arterial or venous samples. The pH values could then be calculated by the use of the familiar Henderson-Hasselbach equation. Although indirect, this method of calculation rests upon a sound basis. Behnke reports the following findings in the case of 9 dogs breathing oxygen at about 3.8 atmospheres:

1. In all cases the venous oxygen content was equal to or greater than the oxygen capacity indicating that the entire metabolic oxygen was supplied by physically dissolved oxygen. That is to say, there was no reduction of the hemoglobin and the mechanism under consideration was operative.

2. The arterial P_{CO_2} was either the same (7 observations) or slightly lower than when breathing air. This is proof that carbon dioxide *transport* is not interfered with.

3. The venous P_{CO_2} was significantly higher than the controls breathing air by an amount averaging 6.5 mm. Hg greater than the controls.

4. The pH of the arterial blood was unchanged whereas that of the venous was less but by a small amount averaging about 0.03 unit.

Behnke concluded from these data that this unique form of CO_2 acidosis expected on physico-chemical grounds does occur but that it is slight, and he dismisses this loss of the dual function of hemoglobin from consideration as a causal factor in oxygen poisoning.

The reasons given by Behnke (somewhat extended by the authors) are: (1) the familiar symptoms of CO_2 acidosis do not resemble remotely those of oxygen poisoning; (2) the changes observed are slight; (3) according to the theory maximal oxygen poisoning should be reached at 2.6 atmospheres, since at this pressure the dual function is completely lost. But Behnke emphasized that oxygen poisoning frequently does not develop promptly at pressures of 3 to 4 atmospheres despite this complete loss. Only 2 out of 9 of his dogs had convulsions even when the hemoglobin of the venous blood was completely saturated. Moreover, Behnke and others have reported that at higher pressures (4 to 6 atmospheres) toxic symptoms are severer and occur sooner than at lower pressures (see section on Tolerance).

One may conclude from the evidence at hand that the changes in the blood expected on physico-chemical grounds do occur when excess oxygen is inhaled, but that they play little or no rôle in the picture of oxygen poisoning.

Enzymatic Systems. The conviction is growing that the action of oxygen is upon the enzyme systems of cells resulting in reversible or irreversible changes such that serious impairment of essential cellular metabolic functions results. The idea goes back at least to Paul Bert's time: Although he found that pressures of oxygen up to 15 atmospheres had no influence upon the activity of such enzymes as salivary diastase, pepsin, invertase, emulsin and myrosinase, he did show that a strip of beef muscle suspended in oxygen at similar pressures showed diminished oxygen and CO_2 metabolism compared to controls under normal conditions. He also found that putrefaction was delayed or inhibited by high oxygen pressures.

The literature on the action of high oxygen upon enzymes may for convenience be discussed in two parts: (1) scattered, relatively non-systematized observations showing the effect of oxygen upon sundry enzymes; (2) more or less systematized studies upon one enzyme forming the basis of an hypothesis explaining oxygen poisoning. These latter are discussed in connection with the hypotheses proposed.

The enumeration of the scattered studies follows:

Confirming Bert's observation on enzymatic autolysis are the studies of Bailey *et al.*[13] and also Laquer.[117] McCance,[129,130] however, found that the effect of aërobic autolysis was mainly upon urea formation which was diminished under oxygen. Meyer[131] showed that the ability of mouse brain homogenates to oxidize guaiacum in the presence of hydrogen per-

oxide was greatly diminished by a preliminary exposure to 4 atmospheres of oxygen for 4 hours. Shapiro and Wertheimer[150] reported that fatty acid dehydrogenase demonstrated by the Thunberg technique in rat tissue is peculiarly susceptible to short exposures to oxygen. Marks,[123,124] and Marks and Fox[125] found that the catalase activity of extracts from marine animals and plants compared to anaërobic controls is significantly diminished by prolonged exposure (1 to 2 days) to air or oxygen. However, oxygen inactivation of catalase is very slow (20 days for complete inactivation at 25° C.).

One difficulty in interpreting the effects of oxygen upon enzymes is illustrated in the case of arginase and urease. Both of these enzymes in impure preparations are inactivated with more or less rapidity by exposure to oxygen, but when purified the inhibiting action is much less.[80,81,93,116,146] In purification, the removal of catalytic amounts of heavy metals—which may catalyze the oxidative inactivation of enzymes—must be borne in mind as a possible cause for their increased resistance.

Gale[87] found that formic dehydrogenase from *B. coli* was inactivated by oxygen. The inactivation was shown not to be due to the production of H_2O_2, oxalic acid or formaldehyde.

Lehman[119] studied the effect of oxygen pressures and pH upon succino-oxidase activity. In general maximum activity was found at comparatively very low oxygen tension, varying somewhat with pH but averaging 49 to 50 mm. Hg. At higher tensions the activity decreased. Bohr and Bean[45] found that preliminary exposure of succino-oxidase from pig heart to oxygen at 5 to 7 atmospheres decreased the subsequently measured activity (as measured by the Thunberg method) from 10 to 50%. No restoration of activity occurred after standing in air. In connection with these examples of the inactivating action of oxygen upon enzymes, attention is called to the point made by Brooks[52] that oxygen may be both inhibitor and reactor; in certain inorganic as well as biochemical reactions it is known to play this dual rôle.

In the following discussion of hypotheses of the toxic effect of oxygen upon enzymatic action, the names given to the hypotheses are those of the Reviewers. In most cases the original work was not done as a primary study of oxygen poisoning. The Reviewers have placed their own interpretation upon the experimental data, but have been careful to distinguish in their discussion their own from the original authors' opinions.

The "Active Oxygen" Hypothesis. Libbrecht and Massart[121] reported studies on succino-dehydrogenase. They constructed a pressure chamber to contain a manometric apparatus of the Warburg type. Small motors in the pressure chamber controlled from the outside manipulated the apparatus. To prevent burning out of the motors, the chamber was filled with air or nitrogen at high pressure, but the respiratory vessels proper, being connected to small balloons containing oxygen, were filled at high pressures (5 atmospheres) upon elevation of the pressure in the chamber. In the case of a freshly prepared succino-oxidase system, the authors found that 5 atmospheres of oxygen completely stopped oxygen uptake in the presence of succinate. That the true dehydrogenase activity was blocked was shown by the fact that the further addition of methylene blue did not restore oxygen uptake. However, aged preparations, or those treated with cyanide to inactivate the cytochrome oxidase system, were unaffected by high oxygen pressure since with succinate such preparations took up oxygen actively provided methylene blue was added to replace the lost cytochrome system as oxygen acceptor. From these

observations, Libbrecht and Massart concluded that molecular oxygen *per se* is non-toxic but that the cytochrome oxidase system together with high oxygen inactivates irreversibly the succino-dehydrogenase by "l'oxygène actif." The term "l'oxygène actif" is too vague a conception for current acceptance. Perhaps the hypothesis could be re-framed by stating that the cytochrome system together with oxygen at high pressures oxidizes the succino-dehydrogenase to an inactive form. This unique hypothesis of oxygen poisoning receives no support from the experimental work of Stadie and associates.[161]

Hypothesis of Reversible Oxidation of Ferrohemochromogens. This well-substantiated and interesting hypothesis of oxygen poisoning centers around the enzyme, hydrogenase.

Hydrogenase catalyzes the exchange reactions:

$$\tfrac{1}{2}H_2 = H^+ + E$$
$$2H_2 + O_2 = 2H_2O$$
$$H_2 + CO_2 = HCOOH$$
$$H_2 + X = H_2X$$

It is present in a variety of bacteria and algæ, but not in mammalian tissue. Stephenson and Stickland,[162] who first described the enzyme, observed that it was inactivated by molecular oxygen, as did Wieland and Pistor,[169] Claren,[71] and Gaffron.[86] Wilson, Lee and Wilson[171] studying the hydrogenase activity of azotobacter revealed a miniature picture of the oxygen problem in mammals. As the oxygen tension rose from low levels the activity of the hydrogenase system increased rapidly since oxygen was playing the essential rôle of reactant. But an optimal oxygen tension was soon attained beyond which a toxic zone was reached wherein activity diminished and finally ceased.

It is upon the interesting and important experiments of Hoberman and Rittenberg[102] that the hypothesis under discussion is based. Using deuterium to measure the activity of the hydrogenase from *proteus vulgaris* they found that the inactivation by oxygen was completely reversible. Reversal could be brought about slowly by re-equilibration with deuterium, and rapidly by reducing agents (hydrosulfite), or biologic agents such as glucose, pyruvate, succinate, formate, or fumarate. The authors concluded that hydrogenase is an iron hemochromogen which can exist in either the reduced or oxidized state. Oxygen inactivation is due to the formation of inactive ferrihemochromogen; reversal of inactivation is brought about by reduction to the active ferrohemochromogen; rapidly by reducing agents, and slowly by deuterium. In further support of this conclusion is the fact that cyanide inactivates under aërobic but not under anaërobic conditions. This was attributed by the authors to the well-known fact, as in the case of peroxidase and catalase, that reduced iron hemochromogens do not combine with cyanide. This attractive and well-substantiated hypothesis of the mechanism of oxygen inactivation of an enzyme cannot, however, explain all toxic action of oxygen. It is even impossible to conclude that all iron or metallo-hemochromogens will react similarly, since in unpublished experiments Stadie, Riggs and Haugaard[161] have found that catalase, a hemochromogen enzyme, is resistant to prolonged action of oxygen at high pressures.

Hypothesis of Action of Oxygen upon Co-enzymes: Glyoxalase. This enzyme catalyzes the dismutation of methyl glyoxal to lactic acid and is abundantly present in mammalian tissue. Reduced glutathione functioning as a co-enzyme is necessary for its action. Jowett and Quastel[110,111]

showed for the glyoxylase of human red cells and rat tissues a progressive inhibition of enzymatic activity in the presence of oxygen which they attributed to the formation of the inactive oxidized form of glutathione.

This appears to be the only known instance of an enzyme system inhibited by the action of oxygen upon the co-enzyme. However, the case of cysteine and carboxypeptidases studied by Irving, Fruton and Bergmann[106] (see *infra* under Formation of Enzyme Inhibitor by Molecular Oxygen for discussion) is also properly discussed here. Cysteine, according to these authors, forms a complex with the enzyme which thereupon acquires activity. The oxidation of the cysteine by molecular oxygen to cystine results in inactivation.

Hypothesis of Inactivation by Oxidation of Activating Metal in Enzyme. This possibility is illustrated by the studies of Warburg and Christian on yeast zymohexase.[167] The enzyme is an important one in carbohydrate metabolism since it catalyzes the splitting of hexose diphosphate into 2 molecules of triose phosphate. The enzyme requires free reduced Fe, Cu, or Co for activation. The action of oxygen is through cysteine in the following way: in the presence of a small amount of cysteine molecular oxygen oxidizes the metal, in which state it is no longer activating. In the case of zinc, which also activates the enzyme, but exists only in one valence state, oxygen and traces of cysteine are without influence. A second mechanism of inactivation by cysteine must be mentioned although not related to oxygen action: an excess of cysteine either anaërobically or aërobically inactivates because of complex formation removing the activating metal.

However, the possibility is remote that this particular mechanism of oxygen action on important carbohydrate metabolic processes is a factor in oxygen poisoning in mammalian organisms, since the zymohexase prepared from muscle is completely independent of metals for activity and in consequence cysteine and oxygen are without influence upon its activity. However, as a prototype, similar oxygen-enzyme interaction may exist in mammalian tissue although the Reviewers have found no references to such.

Formation of Enzyme Inhibitor by Molecular Oxygen. An interesting form of oxygen inactivation is that discussed by Irving, Fruton and Bergmann[107] in the case of swine kidney and beef spleen carboxypeptidases. Experimentally they used the dipeptide carbobenzoxyglycylphenylalanine as substrate and studied its rate of hydrolysis. Cysteine is required for the activation of these enzymes. Oxygen may inactivate by oxidizing the cysteine to cystine which no longer activates (*v. supra*), similar to the case of glyoxalase. But another type of inactivation may result by the action of molecular oxygen, according to the authors. In the first case, if the enzyme was preliminarily equilibrated in the presence of oxygen with cysteine before the addition of the substrate, a diminished but constant reaction rate was obtained. In the second case, if the preliminary equilibration period was omitted, the aërobic digestion began at almost as high a rate as the anaërobic digestion, but this rate diminished to the same low but constant rate as that observed in the first case. This inhibition, the authors concluded, was not due to oxidation of cysteine, which was present in excess and remained essentially unchanged, but cysteine reacted in the course of the incubation with an unknown component of the crude swine kidney extract to form an inhibitor for the carboxypeptidase. Swine kidney pepsinase which requires no activator (*i. e.*, cysteine) was not inactivated by oxygen. These experiments not only illustrate an interesting possible mechanism of oxygen inhibition of enzymatic systems,

but also show that a sulfhydryl activator such as cysteine may react in two ways with molecular oxygen to influence enzyme action: (1) by being itself oxidized and thus losing its function as an activator; and (2) by reacting together with precursors to produce enzyme inhibitors.

Action of Oxygen Upon the Sulfhydryl Enzymes. Recently there has been a renewed interest in the so-called sulfhydryl enzymes. These enzymes are assumed to be active or inactive depending upon the state of the sulfur as a constituent part of the enzyme molecule. Two possibilities are recognized: (1) The sulfur exists in the reduced or sulfhydryl form, designated commonly as EnSH. In most cases (insulin is an exception) the enzyme is active in this state, hence the term, sulfhydryl enzymes. (2) The enzyme may contain the sulfur in the oxidized or dithio form, usually designated by the symbol EnS:SEn, in which condition (excepting insulin) the enzyme is inactive. The transformation of one state to another is in most cases reversible and may be brought about on the one hand by oxidizing agents such as porphyrindine, iodine, iodosobenzoate, oxidized glutathione, iodoacetamide, and so forth, or, on the other hand, by reducing agents such as cysteine, reduced glutathione, thioglycollic acid, hydrosulfite, HCN, H_2S, etc. Reversible inactivation may also be brought about by the use of certain mercaptan reagents, *viz.:* cuprous oxide, mercurials such as chloromercuribenzoic acid, and organic arsenicals which presumably react with the $-SH$ group producing mercaptides according to the equation: $EnSH + X = EnS{\cdot}X$.

The possibility that oxygen at ordinary or high pressures acts as the oxidizing agent to produce enzymatic inhibitions by this mechanism is obviously real, and indeed there is experimental evidence in the case of succino-oxidase that such is the case. This hypothesis, therefore, becomes important in the discussion of the mechanisms of oxygen poisoning.

For discussion of the literature on the sulfhydryl enzymes, reference is made to the review article of Hellerman[92] and the recent articles of Barron and Singer[16] and Potter and DuBois.[141] Only that portion which is pertinent to the subject of oxygen poisoning will be discussed here.

Hellerman[92] reported that urease and arginase—two sulfhydryl enzymes—are inactivated by oxygen but the inactivating action of oxygen is less on the purified enzyme presumably because catalytic copper was removed. Since succino-dehydrogenase is the best studied enzyme in relation to oxygen effects, a more extended discussion is in order. Hopkins and Morgan[104] showed that succino-dehydrogenase, whose action is independent of a co-enzyme, is completely inactivated by the addition of oxidized glutathione. Upon further addition of reduced glutathione or cysteine, complete reactivation is observed. Hence, they concluded that succino-dehydrogenase is a sulfhydryl enzyme and that activity is dependent upon the reduced form—EnSH. In later studies, Hopkins *et al.*[105] made the important observation that malonic, fumaric and succinic acids protected the enzyme from the oxidizing action of the glutathione. Potter and DuBois[141] studied in detail the mechanism of the catalytic action of succino-dehydrogenase. They agreed with Hopkins and Morgan that the action is dependent upon the presence of the $-SH$ groups as constituent parts of the enzyme molecule. They picture the enzyme as being in an equilibrium state between oxidized and reduced form, *viz.:* $EnSH \rightleftarrows EnS + H$.

Alternate oxidation by the cytochrome oxidase system and reduction by substrate explains the mechanism for the oxidation of succinic acid. They further suppose that the active $-SH$ groups are situated between two "affinity" points which combine with the two carbonyl groups of

the succinic acid to hold it for the duration of the action of the oxidative process. Malonic acid also combines with these two points and "covers" the active center —SH group, thus protecting it from the oxidative action of oxidized glutathione or other oxidizing agents. The inactivating action of high pressure oxygen upon succinodehydrogenase has already been discussed. That the mechanism of inactivation is similar to that of glutathione is indicated by the fact that the oxygen inactivation is reversible in part by reduced glutathione, but more significantly by the fact that malonate protects completely.[161]

Whether the inactivating action of high oxygen by this mechanism is a universal manifestation with sulfhydryl enzymes remains to be determined by further experimentation.

Oxidation-reduction Potential of Environment on Enzyme Activity. That enzyme activity may be influenced by non-specific effects such as change of the oxidation-reduction potential of the medium is indicated in the recent work of Sizer and Tytell,[156] and Sizer.[155] They altered the oxidation-reduction potential of their media by the use of a variety of poisoning agents over a wide range and found that the activity of crystalline urease and yeast invertase was a continuous function of the potential. Urease showed a maximum activity at +0.15 volt; invertase showed a constant activity from −0.27 to +0.60 volt, above which it fell off sharply.

Whether or not the oxidation-reduction potential of mammalian tissue can be altered by high pressures of oxygen is unknown; but the possibility that such a mechanism may be operative in oxygen poisoning is mentioned for the sake of completeness.

Summarizing the data on enzymatic systems, the likelihood is increasing that the toxic action of high pressures of oxygen will be explained in the light of inhibitory actions on enzymes with resultant severe disturbances of essential metabolic cellular reaction. The evidence in the literature is scanty and non-systematized, but is sufficient to suggest the following possible modes of action of oxygen: (1) Oxidation of a co-enzyme to the inactive oxidized form. (2) Oxidizing activating sulfhydryl compound. (3) Oxidizing active —SH groups of enzyme molecule proper. (4) Oxidation of metallo-hemochromogen to inactive oxidized form. (5) Oxidizing activating metal constituent. (6) Formation of inhibitor from precursor other than the enzyme. (7) Inhibition of enzymatic activity by changing oxidation-reduction potentials of medium.

Summary. The literature on the subject of oxygen poisoning is reviewed under the following headings: (1) Symptomatology, (2) Pathologic Anatomy, (3) Blood, (4) Circulation and Peripheral Vessels, (5) Metabolism, (6) Blood Gas Equilibrium, (7) Influencing Factors, (8) Tolerance, Adaptation and Oxygen Therapy, (9) Oxygen Aero-embolism from Rapid Decompression, (10) Isolated Surviving Tissue, (11) Neoplasia, (12) Microörganisms, (13) Dual Function of Hemoglobin, and (14) Enzymatic Systems.

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NEUROLOGY AND PSYCHIATRY

UNDER THE CHARGE OF

GEORGE S. JOHNSON

PROFESSOR OF PSYCHIATRY, STANFORD UNIVERSITY, SAN FRANCISCO, CALIF.

AND

FRANKLIN G. EBAUGH*

UNIVERSITY OF COLORADO, SCHOOL OF MEDICINE, DENVER, COLO.

RECENT CONTRIBUTIONS TO WAR NEUROLOGY

BY ALBERT J. LUBIN, M.D.

DIVISION OF NEUROPSYCHIATRY, STANFORD UNIVERSITY SCHOOL OF MEDICINE, SAN FRANCISCO, CALIF.

RECENT literature in the field of war neurology has been largely devoted to the following topics: (1) head injuries, including those resulting from blast; (2) peripheral nerve injuries, including the subject of causalgia and related states; (3) meningococcic meningitis, which has recently risen to epidemic proportions in both military and civilian life; and (4) the use of the electroencephalogram as related to war.

While subjects of spinal cord injury, heat stroke and tropical diseases involving the nervous system may properly be considered here, the paucity of recent contributions has not justified their inclusion. Neither are subjects of neurologic interest in the sphere of aviation medicine included in this review.

Head Injuries. Traumatic disorders of the head may be divided into two general groups, (*a*) closed injuries and (*b*) open injuries. According to Symonds[32] closed head injuries include all trauma without direct penetration of the dura. In this type of injury symptoms are usually those of generalized cerebral disturbance with signs of disordered mental function. Surgical intervention is seldom required except with delayed onset of neurologic signs and increase in mental disability. Such manifestations are presumptive evidence of epidural or acute subdural hemorrhage and exploration through 4 burr holes in the skull is indicated, providing the patient's general condition permits. Of the cases of closed head injury admitted to the hospital, 60% returned to duty. The incidence of post-traumatic epilepsy is small when compared with penetrating wounds. Denny-Brown[11] has also discussed the principles of treatment of closed head injuries. Concussion, contusion, and hemorrhage may occur either

* Now on active service.

independently or in combination. As a result, clinical manifestations are varied and difficulties in therapy are encountered. Indications for treatment are discussed as follows: (1) Rapid increase in coma or in signs following initial improvement is indicative of an extradural hemorrhage, and immediate surgical intervention is indicated. When retrogression is slow, acute subdural hemorrhage or subdural hygroma is a likely possibility. According to Denny-Brown, the results of surgery in these latter conditions are poor. Conservative management is usually indicated, as the subdural hemorrhage of hygroma is frequently of secondary importance to concussion of the brain. Increase in psychomotor activity is rarely observed when a compressing mass is of significant size. (2) An increase in spinal fluid pressure accompanied by deepening coma is an indication for exploration. However, a moderate increase in pressure without an increase in the depth of coma is of no especial significance. Denny-Brown believes that increase in pressure is most frequently due to impaired venous drainage, not cerebral edema. (3) In the group of patients in which symptoms and signs decrease, increased pressure and localizing signs are absent and the electroencephalogram contains diffusely abnormally slow activity, surgical intervention is not indicated.

In cases of cerebral concussion, supportive measures, rest and the use of oxygen will usually lead to recovery.[10] In the unconscious patient the importance of keeping the respiratory passages open cannot be minimized. He should be placed on his side or even on his abdomen with the head turned toward one side, thus insuring easy breathing and the escape of saliva. Mock and Mock[24] advocate the transportation of these patients in the prone position; suction apparatus should be available. Dependent drainage is of help in some patients, according to Peet.[24] Utilizing animal experimentation, Faulkner[14] points out that in cases of head injury with bleeding from the mouth and nose, respiratory difficulties may be the result of blood entering the trachea and lungs rather than due to involvement of the respiratory center as is commonly assumed. Morphine is avoided by most observers because of its depressing effect on the respiratory center and its tendency to increase intracranial pressure.[24,27] Horrax,[21] however, states that small doses may be given preoperatively if the patient is not unconscious or extremely drowsy. Mock and Mock[24] believe that all types of disturbing influences should be avoided during the first 6 hours, which is the most critical period. These include suturing of wounds, oversedation, the use of antitetanus serum or even transferring the patient to a better room. The patient should be placed between blankets, heat applied and oxygen given as indicated.

The use of dehydration and spinal drainage in cases of head injury associated with increased spinal fluid pressure is still a controversial subject. Denny-Brown,[11] for instance, maintains that dehydration is generally of no benefit and may be of harm. Mock and Mock have surveyed the management of 6544 consecutive cases of head injury throughout this country and feel that the moderate use of dehydration and spinal drainage have proved themselves to be of value. Symonds[32] advocates the use of lumbar puncture as a guide to therapy. If the pressure is 250 mm. or more, withdrawal of fluid is indicated. When headaches associated with increased pressure are not alleviated by raising the head, hypertonic solutions by mouth or rectum may be employed. Rowbotham[31] feels that the determination of the spinal fluid pressure and the presence of subarachnoid bleeding by means of lumbar puncture is important in the management of the acute brain injury. Lumbar puncture should be per-

formed after 12 hours and fluid removed until the pressure reaches 50 mm. of water. If the initial pressure is high, the pressure should be repeated as often as every 4 hours. Rowbotham advocates the moderate use of 50 % sucrose solution and magnesium sulfate enemas as dehydrating agents. Spinal drainage without dehydration is used when subarachnoid bleeding has occurred. Rogers[29] prefers the use of magnesium sulfate rectally rather than intravenous methods. Magnesium sulfate should not be employed intravenously. Turner[34] reports success with the use of concentrated serum as a dehydrating agent.

Cairns and Holbourn[7] describe the advantages of the use of crash helmets by motorcyclists. Head injuries have occurred in 41 % of a series of motorcycle accidents. The crash helmet, made of either vulcanized rubber or preferably compressed wood pulp, tends to spread the blow over a wide area and prevent lacerations, fractures and brain contusions. It also spreads the blow over a longer period of time, thus diminishing the rate of deceleration of the brain.

Horrax[21] divides head injuries with penetration of the dura into: (1) gutter wounds, in which the missile has made a furrow through the scalp and caused fragments of bone, hair and clothing to penetrate into the brain; (2) simple penetrating wounds, which also carry débris into the brain; (3) penetrating wounds from multiple, usually small, fragments which are especially common in this war; (4) perforating injuries, with débris scattered along the track; and (5) injuries involving air sinuses. In the first 2 types contaminated scalp and contused brain are removed. A soft rubber catheter is inserted into the track in order to locate fragments. The area is cleaned by suction. Experience in this war has shown that multiple, small penetrating fragments are best left alone. Perforating or through-and-through injuries are usually fatal. In survivors, there is usually little to do. Limited débridement may be indicated, and accessible bone fragments should be removed. Injuries involving air sinuses are especially serious because of the continued source for infection. Closure of the dura is imperative when possible. If this is not possible, muscle implants or gauze packs may be used.

Experience has shown that innocent-looking penetrating scalp wounds are potentially dangerous and require careful attention. Botterell and Jefferson[6] state that one-quarter of the air raid casualties admitted to an English hospital had scalp wounds. Blood was found in the spinal fluid in one-fifth of the cases of wounds apparently confined to the scalp. Emergency treatment, according to Craig,[10] should consist of shaving the scalp, cleansing the wound with a minimum of manipulation, introduction of sulfanilamide powder and application of a sterile dressing. Extensive operative procedures should be delayed until adequate facilities are available, a neurologic examination has been made and Roentgen rays of the skull taken. With the use of sulfonamides, wound closure may be delayed from 24 to 48 hours.

Miller,[23] citing his experiences in this war, strongly condemns the failure to perform primary suture of head wounds. The open treatment is frequently followed by intradural infection and should be used only in cases of long standing with obvious infection. In some cases uncomplicated healing is obtained with wounds older than 48 hours. He advocates the transfer of patients with head injury to a center where competent care is available. Bone or metal fragments that cannot be found and sucked out without the use of Roentgen rays should be left alone. Because of this he states that Roentgen rays are not always essential prior to the operation.

Cloward,[9] treating the first American casualties of this war in Hawaii, notes that nearly all head injuries were associated with compound depressed fractures of the skull. These were caused by sharp, irregular shell fragments apparently traveling at high rates of speed; as a result many of them penetrated into the brain substance. Cloward points out that most of the patients with penetrating wounds did not lose consciousness. This tends to confirm the experimental studies of Denny-Brown and Russell[13] on concussion. They have shown that the movement of the brain must be accelerated at least 28 feet per second in order to produce unconsciousness. Cloward expresses the belief that small fragments enter the skull at such a high rate of speed that generalized acceleration of the brain is not produced. As Kennedy[22] has also noted, when consciousness is lost as a result of this type of trauma it is by reason of damage to extensive areas of brain and not merely the result of concussion. The prognosis in unconscious patients with penetrating head injuries is therefore poor. Among the patients so injured in Hawaii, all of those admitted to the hospital died within a few minutes to 12 hours. Cloward mentions a few important points upon which most authors agree in the treatment of penetrating wounds of the head: "1. The optimum time to operate is within 6 hours after the injury, but wounds which have gone untreated as long as 48 hours should be treated as fresh wounds and closed without drains, unless there is an obvious infection. After 48 hours the wound is treated as a brain abscess with open drainage. 2. All metallic foreign bodies should be removed as soon as possible, because they lead to secondary abscess and epilepsy. 3. The defect in the dura mater should be closed completely in clean wounds with fascia or periosteal grafts. 4. Generous use of sulfanilamide not only in the scalp and skull wound but in the missile tract in the brain is recommended. Sulfanilamide is preferred because it has been found to be much more rapidly absorbed into the blood stream from an open wound than any of the other sulfonamide drugs."

Munro[26] emphasizes that operation should not be performed unless the general condition is satisfactory. Treatment for shock is begun immediately and débridement is delayed until such treatment is effective. Munro condemns shaving and washing the wound until the patient is ready for operation. Diagnosis is made by palpation through the wound. The wound is then covered with a sterile gauze dressing and further manipulation is avoided until the patient is ready for operation. Complete débridement should be performed within 48 hours after the injury or else should be delayed for 6 to 8 months. Suction rather than irrigation is the most effective method of wound cleansing; irrigation tends to spread infection. Drainage is not necessary in any wound in which débridement has been properly performed. While Munro recommends the use of sulfonamides, he believes that they should be used only as an adjunct to proper surgical treatment. Bone or metal fragments lodged in the brain should be removed if accessible. However, if removal necessitates further damage to normal brain, non-interference is the best policy. Electromagnets have been utilized in the removal of metal.[9] Money and Nelson[25] feel that it is more important to remove bone fragments and inorganic débris than metallic foreign bodies. The dura mater should be closed in order to prevent the development of herniation of the brain, cerebrospinal fluid fistula or aërocele. Dural defects may be repaired by use of a periosteum patch obtained from the overlying skull.[9]

The mortality rate of 60% in cases of head injury during the early stages of the first World War, which was later cut in half by Cushing's improve-

ments in surgical technique, is evidently becoming even smaller in the present war. This is largely made possible by the local and systemic employment of the sulfonamides. Carmody,[8] for instance, reports that all of 12 patients with major gunshot wounds of the head recovered in spite of the fact that 10 of them had severe infections on admission to the hospital. This recovery is attributed in large measure to the local application of sulfanilamide powder supplemented with parenteral sulfadiazine. Watt and Alexander,[35] however, report the frequent occurrence of generalized or focal epileptic seizures following the local application of sulfathiazole to the brain. The local use of this particular drug is therefore contraindicated. No such complications have been encountered with sulfanilamide, sulfapyridine or sulfadiazine.

A variety of substances have been utilized in the repair of bony defects in the skull. Bone grafts have been preferred, but various types of metal plates, celluloid and cartilage have been used. Objections have been raised to all of them. The discovery by Venable and Stuck that vitallium, an alloy of cobalt, chromium and molybdenum, produces no tissue reaction and has tremendous tensile strength has been utilized by Geib[17] and Beck.[5] Geib reports the use of vitallium in the repair of skull defects using casts of metal previously designed to fit the defect. Beck has modified the procedure by employing stock plates that can be used without preliminary casting. More recently Pudenz[28] and Fulchar[16] have reported the use of tantalum. This metal is said to be non-irritating, easily malleable, inelastic, usable as extremely thin plates, and non-absorbable.

Blast Injuries. The effects of blast on the organism are profound. These are mainly in the lungs and, particularly in the case of immersion blast, the gastro-intestinal tract. There are, however, relatively few reports on the effects of blast on the central nervous system. Most observers have felt that such effects are not conspicuous and are of secondary importance to those in the chest and abdominal cavity.[15] Ascroft[4] describes numerous petechial hemorrhages in the cerebral cortex of a person dying from the nearby explosion of a hand grenade. Zuckermann finds that intracranial hemorrhages are confined to the pia and the tela choroidea in experimental animals subjected to blast.

Anderson[3] has discussed the psychiatric disturbances found in 8 persons exposed to the detonation of high explosive bombs. He expressed the belief that impairment was due to structural changes in the brain, but that constitutional or psychogenic factors may have coëxisted and deserve consideration in the plan of therapy. In general, periods of unconsciousness following the blast were absent or mild. Impairment of memory was the most characteristic feature. Lack of concentration, forgetfulness, lability of affect, apathy, transient aphasia, depression and dementia were noted.

Abbott, Due and Nosik[1] have observed 10 cases of subdural hematoma or subdural effusion of spinal fluid following blast injury. The patients present a history of exposure to severe concussion, loss of consciousness for a period varying from a few minutes to several days, persistent headaches, memory loss and irritability. Neurologic signs are not conspicous. "The most pronounced symptoms are persistent headaches, usually generalized (increased on exertion and often nocturnal), a history of coma, syncope or convulsions which did not exist prior to the blast, and a definite departure from a stable personality." When the lesion is suspected, pneumoencephalography is indicated. "If a hematoma or effusion is present in the subdural space, a characteristic filling defect is observed over the

cerebrum with an occasional distortion of the ventricular system." The total protein of the spinal fluid may be increased. Removal of the entrapped fluid led to prompt recovery. Five of the cases were bilateral.

In another report[2] the same observers have described the psychiatric picture in greater detail and the use of psychologic testing in diagnosis. Two syndromes are distinguished. The first and more common is one in which pronounced retardation in intellectual activity and interpersonal relationships occurs. The person tends to be dull and lacks "push." The second syndrome is seen in the more chronic cases; its most striking feature is impairment of inhibitions, with euphoria, motor restlessness and emotional lability. The Rorschach test and especially the Shipley-Hartford Retreat test for intellectual impairment[30] have been useful adjuncts in arriving at the diagnosis. In the latter procedure a vocabulary test, not easily affected by structural disease, is contrasted with an abstraction test, which is readily impaired by such changes. The authors conclude that "intellectual impairment in blast cases with or without positive neurologic findings is an indication for a pneumoencephalogram."

Blast injuries following underwater explosion present a particular problem. Experiments by Greaves *et al.*[19] illustrate the danger of having the head below the surface during the blast. Whereas guinea pigs swimming on the surface had no macroscopic brain lesions, 4 guinea pigs with heads submerged, all had extensive epidural and subdural hemorrhages. One had a skull fracture; 2 had hemorrhages extending into the spinal canal. Hamlin[20] describes the clinical syndrome in immersion blast injuries as consisting of clouding of the sensorium and diminished reflex activity commensurate with the severity of the visceral and pulmonary injuries. In some cases red cells or an increase in the total protein may appear in the spinal fluid. The mechanism of the production of neurologic symptoms is still in the theoretical stage. Hamlin suggests that the blast wave may be transmitted to the neuraxis so that cerebral acceleration is produced as in cerebral concussion. It is probable, however, that the neurologic difficulties are due more frequently to transient ischemia from the rapid shift in vascular reservoirs. He notes that all survivors within the danger zone of an underwater detonation will have had their heads above the water when the compression occurs. Pain in the limbs, testicles, abdomen and chest is thought to be the resultant of forces acting on the peripheral and autonomic nerves.

Chronic Effects of Brain Injury. Denny-Brown[12] has reviewed his experience with 400 cases of prolonged disability following head injury mainly as the result of the war. Psychoneurotic symptoms were found to be more frequent problems than "organic" defects; 262 cases were suffering from psychoneurosis; 22 from traumatic epilepsy, and 116 from other organic defects. Overemphasis is too frequently placed upon either the structural or the emotional factors. Satisfactory therapeutic results require attention to both factors. Denny-Brown expresses the belief that psychoneurotic symptoms may result from structural damage following severe injury. These symptoms may also be a consequence of attempting to adjust to a pre-traumatic level in the presence of intellectual impairment. When no evidence of severe injury is obtained, one can usually elicit a history of previous emotional instability. In cases of concussion, the duration of amnesia following the trauma is the most reliable indicator of cerebral damage.

Important factors in prognosis, according to Symonds,[32] are: (1) the pre-traumatic constitution, as gauged by the family and personal history;

(2) age; (3) duration and degree of mental disorder following the injury; (4) duration of traumatic amnesia; and (5) the particular situation of the individual, including his desire for return to duty, anxiety regarding future capacity and compensation factors. Reassurance, occupational therapy and graduated increases in activity are useful therapeutic agents.

Symonds and Russell[33] state that the prognosis in 718 cases of chronic head injury admitted to a military hospital because of unsatisfactory progress was twice as poor as in their acute cases. They believe that a greater predisposition to mental disorders existed in this group. The prognosis in 111 members of the Royal Air Force with acute and chronic head injuries was 4 times as good as in the entire series. These men were highly selected as far as mental and emotional disorders were concerned.

The recent publication of Kurt Goldstein,[18] "Aftereffects of Brain Injuries in War," emphasizes the important problems that will be encountered in the care of the more or less permanently disabled cases of head injury. The book is divided into two parts. The first deals with the general, neurologic and mental symptoms and with a variety of testing procedures used to elucidate more obscure defects. The second part is concerned largely with the treatment of aphasic and agnosic disorders and the principles employed in determining subsequent placement and the patients.

Epilepsy, Goldstein notes, is a frequent sequelæ of penetrating skull wounds; grand mal and petit mal attacks occur in 15 to 20% of all patients and other varieties are common. Attacks occurring shortly after the injury are less inclined to become chronic than those appearing after 1 or 2 years. Many of the early seizures are associated with irritative factors that can be eliminated, while late attacks are frequently associated with scarring. He points out the importance of careful perimetric examination in these patients. Visual defects may be due not only to focal lesions of the visual apparatus, but also to diffuse blows; or they may be an expression of abnormal fatigue. Central vision usually improves earlier than peripheral, so that the resultant tubular constriction may be confused with that seen in hysteria. Disturbances in space perception and in color vision are also noted.

Frontal lobe involvement is common. Extreme akinesia with apparent intactness of the memory and other mental functions may be noted; sometimes it is associated with catalepsy. Abnormal postures and disturbances in coördination are observed. Aphasia, apraxia and related phenomenon may be due to lesions in the second and third frontal convolutions. Goldstein describes the mental changes in frontal lobe lesions as an impairment in the capacity of "abstraction;" the patients become more "concrete in their behavior." "The abstract attitude is basic for the following potentialities: 1. Assuming a mental set voluntarily. 2. Shifting voluntarily from one aspect of a situation to another, making a choice. 3, Keeping in mind simultaneously various aspects of a situation. 4. Grasping the essential of a given whole, breaking up a given whole into parts and isolating them voluntarily. 5. Abstracting common properties, planning ahead ideationally, assuming an attitude toward the 'merely possible,' and thinking or performing symbolically. 6. Detaching the ego from the outer world. In all these potentialities, the patients are more or less impaired."

Another group of symptoms arise not directly from the damage to the brain but from "the struggle of the changed organism to cope with the defect and to meet the demands of a milieu with which it is no longer able

to cope." Behavior as a whole therefore differs fundamentally according to the situation. In the face of a situation to which he cannot adapt, the patient may exhibit a variety of mental, emotional and vasomotor phenomena. Goldstein calls this a "catastrophic situation." Numerous psychologic laboratory examinations have been carried out in order to evaluate the mental functions, the general level of performance, the presence of circumscribed mental defects such as aphasia, and to study the working capacity in special kinds of labor. The mere results of tests, such as the intelligence quotient, are ambiguous. The procedure in which the patients arrive at them, however, is of more significance. In the tachistoscopic examination a variety of letters, words, figures, numbers, objects and colors are projected upon a screen for a fraction of a second. One-tenth second, for instance, is ordinarily required to recognize familiar objects. In cases of brain damage the time required to recognize them may be lengthened. "With this plan one can find slowing down of mental processes, disturbances of attention, of memory, of the visual capacity in different directions, disturbances of reading (alexia), of visual recognition of objects (agnosia), as well as defects of the visual field, disturbances of color vision, aphasic symptoms." Ergographic tests are used for the study of fatigue and working capacity. The observation of labor in hospital workshops is of use not only in retraining but also in giving indications of the patient's future work capacity.

The section on therapy is devoted to a detailed discussion of the re-educational training procedures employed in a variety of aphasic and related disturbances. Training is based on the previous careful evaluation of the patient by clinical and laboratory methods. Treatment, in general, is devoted to retraining functional mechanisms, as in childhood, or, in case the brain apparatus is so damaged that former performances are irrevocably lost, by building up substitute mechanisms.

In patients discharged after treatment, Goldstein notes that while but 20% became almost normal workers, only 18% did nothing and earned no money.

Peripheral Nerve Injuries. Injuries to peripheral nerves are an important problem in war medicine. Among 383 injuries treated in the surgical service of an American naval hospital, comprising casualties from the Solomon Islands engagement, there were 30 peripheral nerve injuries.[40] In this group the nerve injuries were frequently associated with compound fractures or with wounds that were not healed sufficiently to allow an anastomosis. Therapy, therefore, was devoted to promoting healing and avoiding contractures rather than nerve suture.

Seddon[52] has suggested a new classification of peripheral nerve injuries: (1) Complete anatomic division (neurotmesis). Prompt surgical intervention is indicated in this group. (2) Complete loss of function without anatomic disturbance of the internal structure (axontmesis). Differentiation between this and the first type ordinarily can be made only by exploration. When no anatomic division is seen, regeneration occurs more rapidly and completely; there should be no interference with the nerve. (3) Transient block (neurapraxia). This group is characterized by partial loss of function which is mainly motor in character, little wasting of musculature, absence of a reaction of degeneration, the presence of paresthesias and relatively rapid recovery of function without surgery.

The character and treatment of peripheral nerve injuries encountered in war has been discussed by Craig.[39] He notes that war injuries present a more difficult therapeutic situation than those ordinarily seen, inasmuch

as tissue destruction is usually greater and suturing is delayed longer. In spite of the new methods of treatment that are being developed at the present time, Craig expresses the belief that severed nerves are best treated by end-to-end anastomosis. If possible, this should be done without tension, hemorrhage, or infection. He advocates the use of local and systemic sulfonamides. "The sulfonamides do not inhibit nerve regeneration but allow for the suture of infected or potentially infected nerves, thus shortening the period which formerly was allowed for the infection to clear." Suturing is ordinarily performed with silk or cotton. Steel and tantalum, which produce no tissue reaction, are proving to be of value. "Nerve grafts are as yet not as efficacious as end-to-end suture, which may be achieved by multiple operations with lengthening of the nerve by wide dissection and primary suture of the separated nerve ends with flexion of the neighboring joints." Craig states that massage and passive motion are useful in keeping the muscles in good condition, but they do not stimulate nerve regeneration. The radial nerve appears to be most frequently involved. Wounds of the median nerve are most often complicated by the development of causalgia. Injury to the brachial plexus is commonly the result of concussion, hemorrhage or stretching rather than laceration. It is therefore best to delay operative procedures with this type of wound in the hope that spontaneous recovery will ensue.

According to Learmonth and Wallace,[46] peripheral nerve injuries are frequently associated with scarring due to tissue destruction and sepsis. They believe that active coöperation between the neurosurgeon and the plastic surgeon is essential to proper therapy. After neurologic examination, the plastic surgeon should obtain a covering of normal skin and subcutaneous tissue for the damaged area. Exploration of the nerve is carried out later.

The rate of nerve regeneration following operation has been calculated by Marble, Hamlin and Watkins.[48] The average rate in cases of ulnar nerve injury was 1.24 mm. daily, with the results varying from 3.6 to 0.58 mm. daily. The average rate in radial nerve injury was 1.09 mm. daily, with a range of from 2 to 0.5 mm. They point out that in nerve injuries associated solely with fractures, nerve suture is seldom indicated.

A variety of new techniques in nerve grafting have been reported in the last few years. Nerve grafting was formerly restricted for the most part to the use of fresh grafts from non-essential nerves of the injured patient. The use of coagulable plasma as a glue to replace ordinary sutures was reported by Young and Medawar in 1940.[55] The plasma was obtained from cockerels and chick embryo was added as the clotting agent. This "fibrogen-rich glue" was placed in the gap between the nerves and formed a bridge across which new axons would grow. Bentley and Hill[37] described the experimental use of transplants from other animals of the species. Tarloff and Benjamin[53] have modified the method of Young and Medawar by using autologous plasma fortified with autologous muscle extract. They believe that this preparation results in less subsequent inflammatory reaction and scar formation.

Weiss and Taylor,[54] in attempting to overcome the difficulties of using preserved nerves as grafts, have devised a method in which the nerves are frozen and dehydrated and then stored aseptically. They found that when the nerves were rehydrated that they assumed their normal histologic appearance and could be successfully grafted into animals of the same

species. They suggest that "banks of assorted nerve sizes stored in the dried condition could readily fill a steady demand."

de Rezende[41] reported the use of peripheral nerves obtained from cadavers in bridging nerve gaps in experimental animals. The first clinical application of this procedure was made by Klemme, Woolsey and de Rezende.[44] The grafts were suspended in a solution of formaldehyde, transferred to alcohol 2 or 3 days before the operation, and immersed in a saline solution ½ hour before the operation. The graft was fixed in place using a 50% acacia glue and an allantoid membrane was placed around it.

Neuroma formation following peripheral nerve injury is commonly attributed to the misdirected and excessive outgrowth of the proximal end of the neurones which are attempting to find a pathway down the distal portion of the nerve. Klemme, Woolsey and de Rezende point out that neuromas will form at both of the cut ends of a nerve although the proximal end is usually larger. The suggestion is made that neuroma formation is due mainly to hemorrhage with scar formation and that the growth of axons distally merely increases the size of the proximal neuroma. They emphasize that operation should be performed immediately after the injury or else after the wound has healed completely. Grafts should be used if there is any tension on the nerve; a graft may be applied later if immediate suture is unsuccessful. They point out that suturing results in laceration of many of the axis bundles and for this reason prefer the use of nerve grafts.

Richter and Katz[49] have described an objective method of mapping out peripheral nerve or sympathetic involvement by measurement of the electrical skin resistance. They have found that skin resistance is higher in denervated areas and that these areas are sharply demarcated with this procedure. It is possible to map out areas affected by peripheral nerve injuries without the active coöperation of the patient and is therefore an advantage in the examination of patients who are unable to coöperate and in cases of malingering. It should be of help in following the progress of nerve regeneration after suture. Richter and Katz state that the apparatus is simple and the method rapid.

Massage, motion and the application of moist heat are ordinarily advocated beginning about 2 weeks after the operation. There is no common agreement as yet on the effect of electrical stimulation in retarding the rate of muscular atrophy following denervation. Guttmann and Guttmann[42] and Hines, Thompson and Lazere[43] have expressed the belief that beneficial effects are obtained. Lavrentjev[45] has stated that heat is the sole means of increasing the rate of nerve regeneration. Billig and Van Harreveld[38] have suggested a new method for the re-innervation of chronically paralyzed muscles such as found in poliomyelitis or peripheral nerve injuries. The method is based on the fact that regeneration of nerve fibers is accompanied by branching. They therefore interrupt the remaining intact nerve fibers of a motor nerve innervating a paretic muscle and depend on the branching during regeneration to obtain an increase in the number of active muscle fibers. The interruption is accomplished by the pressure of forceps without interruption of the nerve sheath and can only be applied to motor nerves which contain few sensory fibers.

Causalgia and Phantom Limb Pain. Pain following peripheral nerve injury or amputation may be expected to be frequently seen in wartime. Causalgia and related states were described in detail by Weir Mitchell in soldiers injured in the Civil War. Little has been added in the intervening years in elucidating fundamental mechanisms involved. Livingston's

publication, "Pain Mechanisms: A Physiologic Interpretation of Causalgia and Its Related States,"[47] reviews the subject and makes provisional attempts to correlate it with recent neurophysiologic and anatomic concepts. The term causalgia ("burning pain") has been used to designate a syndrome which follows peripheral nerve injuries and is characterized by: (1) burning pain; (2) trophic changes, especially glossy skin; and (3) a local rise in temperature. Livingston believes this definition to be too restricted; all 3 are symptoms which follow peripheral nerve irritation and which may occur either isolated or in combination. Moreover, hyperesthesia and sweating are often noted, and coldness is more frequently present than heat. Persistence of the symptoms may result in thinning of the skin, fibrosis and atrophy of muscles, and atrophy of the bones with osteoporosis. He restricts the term causalgia to severe cases characterized by burning pain, employs Homan's term, "minor causalgia," for less severe cases and "post-traumatic pain syndromes" to include cases in which burning pain is not a feature. Occasionally the pain and other symptoms are reflected to the same position in the contralateral extremity as a "mirror image." This mirror image may persist after the original lesion has disappeared. Livingston states, "It is difficult to escape the conviction that some dynamic process has been initiated within the spinal cord that may persist after the original stimulus is withdrawn."

Riechert[51] gives the incidence of causalgia as 2% in a series of 500 cases of peripheral nerve injury. While it usually follows trauma to the sciatic or median nerve, injury to other nerves may give rise to the phenomenon. It occasionally appears after wounds in which no opening of the skin or fracture has occurred. The pain is aggravated by contact with anything that is dry. A central disturbance is indicated by the fact that emotional experiences may precipitate an attack. Gask and Ross's view that pain from peripheral nerve irritation is due to the excessive liberation in the skin of an "H-substance" which increases skin sensitivity is not necessarily disproved by the relief of pain by sympathectomy. Vasodilation following this procedure may in effect assist in washing away the substance.

The term "phantom limb" is applied to the sensation of the persistent presence of a limb after it has been amputated.[47] This sensation may, indeed, be more vivid than when the extremity was intact. Sometimes, severe pain is felt in the phantom limb, most frequently of a burning character. Occasionally, the patient feels as if he is able to voluntarily move the fingers or toes, or they are felt to be in a cramped position. In the presence of phantom limb pain, the stump is commonly cold. Hyperesthesias and sweating may be present. Fibrillary twitches and clonic contractions are observed in some stumps, and they may be increased with emotion.

Bailey and Moersch,[36] in a study of 105 patients, note that phantom limb appears after most amputations (90.5%) but becomes incapacitating in only a few. Among 56 amputations necessitated by trauma, the sensation occurred immediately in 42 and was delayed in the remainder. Symptoms did not become distressing, however, until later. This period averaged 5 years, though as long as 29 years elapsed. Of 15 varieties of surgical and non-surgical therapies none gave anything but transient relief. The authors could find no single theory to explain the phenomenon. They concluded that it probably is of psychogenic origin, possibly a type of "obsession neurosis."

Riddoch[50] believes that impulses from the stump are fundamentally responsible for the sensation. The impulses attempt to maintain an image

of the intact "body scheme" in consciousness. These excitation impulses are subject to inhibitory processes in the central nervous system. If the peripheral excitation is intense and the central inhibitory processes are weakened, painful sensations may result. Thus, ill-health or emotional disturbances may precipitate the painful state by reducing central inhibition.

Livingston, likewise, believes that the fundamental etiologic factor in all of these conditions lies in irritation arising from the traumatized peripheral nerve. In proposing a theory to explain the phenomenon one must take into account the fact that eliminating the focus of irritation does not always abolish the pain. Livingston utilizes the concept of self-reëxciting internuncial pools of neurons in which nerve impulses may continue to discharge after the stimulus has ceased; this type of neuronal arrangement has been suggested by neurophysiologists as an explanation for after-discharge. He summarizes, "An organic lesion at the periphery, involving sensory nerve filaments, may become a source of chronic irritation. Afferent impulses from this 'trigger point' eventually create an abnormal state of activity in the internuncial neuron centers of the spinal cord gray matter. The internuncial disturbance in turn is reflected in an abnormal motor response from both the lateral and anterior horn neurons of one or more segments of the cord. The muscle spasm, vasomotor changes, and other effects which this central perturbation of function brings about in the peripheral tissues, may furnish new sources for pain and new reflexes. A vicious circle of activity is created. If the trigger point is removed early, the process may subside spontaneously. If the process is permitted to continue, it spreads to involve new areas, and tends to acquire a momentum that is increasingly difficult to displace. Perhaps in this stage, even a removal of the original irritant may not be sufficient to establish a cure. But if an important part of the circle of reflexes can be interrupted, the process may subside, and a normal physiologic status is again established. If again the pathologic patterns gain the ascendancy, the repeated breaking of the circle may result in a permanent cure." Therapy should therefore not be delayed. Frequently novocaine injection or surgical removal of the trigger point helps to break up the vicious circle. If this does not succeed, interruption of the sympathetic pathways, either by injection of sympathetic ganglia, periarterial sympathectomy or sympathetic ganglionectomy, may be of benefit. If in turn this approach is unsuccessful, posterior rhizotomy or anterolateral chordotomy may be attempted.

Livingston does not subscribe to the view, held by Foerster and Leriche, that sympathectomy relieves pain by interruption of sensory fibers, inasmuch as experimental evidence indicates that all such fibers pass through the posterior roots. Lewis submits that vasodilation and increased blood flow is the only explanation available at the present time for benefits from sympathectomies. While not denying the importance of the vasomotor factor, Livingston is not completely satisfied with this explanation, feeling that it is but a part of a more fundamental central disturbance.

Meningococcic Meningitis. In a review of the health of the U. S. Army, Simmons[64] states that the army has experienced an outbreak of meningitis which is similar to, though milder than, that in the first World War. A sharp peak of 4.5 cases per 1000 per annum was reached in 1918. A similar abrupt rise to 2.7 has occurred in this war and has been maintained for a longer period than in the first World War. The incidence in the civilian population has increased fivefold over the previous 5 year average.

The army outbreak began at approximately the same time throughout the country and was of more serious consequence in camps with a large turnover. The case fatality rate has decreased from 34% in World War I to the present rate of between 3 and 5%. This is chiefly due to the use of sulfonamides.

Taranto[66] has made an analysis of the symptomatology found in 100 consecutive cases of cerebrospinal fever at a navy camp. The most prominent features were headache (90%), nuchal rigidity (80%), vomiting (64%), petechiæ (64%) and prostration, delirium or coma (38%). He was impressed by the paucity of neurologic findings; the Babinski sign, for instance, was elicited in but 5% of the cases. Only 1 patient succumbed. Sulfadiazine was of inestimable value. In most cases 4 gm. were given by mouth on admission, followed by 2 gm. in 3 hours, 2 gm. in another 3 hours and then 1 gm. every 4 hours until the temperature was normal for 72 hours. Delirious patients were given sodium sulfadiazine intravenously. The average period of therapy was 8 days. Incoming raw recruits were most susceptible to the disease. With the arrival of a new battalion, the incidence increased to a peak in 3 weeks and then gradually declined. Awe, Babione and De Lamater[56] have reported 2 deaths in 50 cases of meningococcic meningitis seen at the U. S. Naval Hospital in San Diego. One occurred 20 minutes and the other 4 hours after admission. Eleven of the patients were comatose and 27 stuporous or irritational. In spite of the severity of the process in most cases, striking improvement was apparent within 24 hours after administration of sulfapyridine. They have emphasized that successful therapy depends upon early diagnosis and prompt use of large amounts of sulfonamides. Meningitis should be considered in every man complaining of cold and headache or backache; lumbar puncture should be performed without delay in any suspicious case. Large initial doses of sulfonamides were considered essential. "The primary dose here has been either 4 or 6 gm. of sodium sulfapyridine intravenously, followed by 2 gm. intravenously every 4 hours. This routine is continued for 3 to 5 days." This was followed by oral administration in 29 patients. Fifteen patients received serum in addition to sulfapyridine. No difference was noted between this group and the remainder who received sulfapyridine alone, except for the high incidence of serum sickness. Newcomber and Frame[62] have also reported 2 deaths in 50 cases and no deaths among those with 12 or more hours of treatment. Parenteral sodium sulfapyridine was used in this group.

Daniels, Solomon and Jaquette[57] have studied 112 patients with meningococcic infection at Fort Bragg, N. C. The incidence of infection increased markedly beginning December 30, 1942, rising to 8 per 1000 per annum for the entire camp and 20 per 1000 annually for unseasoned troops. Of all these cases, 59% were from the group of soldiers with less than 3 months' service comprising one-third of the total camp population. The authors have stressed the importance of viewing the disease primarily as a meningococcic septicemia, as it is only in this way that the diagnosis can be made before meningitis develops. Infection arises in the nasopharynx and invades the blood stream, with localization in the skin, joints, meninges and other body tissues. In 32 cases a diagnosis of meningococcemia without meningitis was made. While upper respiratory infection, headache and nausea were among frequent symptoms of meningococcemia the most characteristic manifestation was the rash. Most commonly it was petechial or purpuric. Ill-defined pink macules and maculopapular lesions were also seen. The initial blood culture was positive for men-

ingococci in 56%; therefore in many cases diagnosis had to rest on clinical grounds. Prompt therapy prevented the advent of meningitis.

In the 80 cases of meningitis, bacteriologic confirmation was obtained in 97.5% of the patients who had not received any sulfonamide previously. On the other hand, no meningococcus was grown in any patient previously given the drug. Only 1 death occurred in the entire group. Sodium sulfadiazine, intravenously, was the drug of choice in cases of meningitis. One group received 0.1 gm. per kg. intravenously as an initial dose and one-half this amount every 8 hours thereafter until it could be retained by mouth. Another group received an initial dose of 0.05 gm. per kg., 0.025 gm. per kg. 4 hours later and then the latter amount every 8 hours. The smaller dosage was equally effective, and the incidence of renal complication from sulfadiazine decreased.

Hill and Lever[60] reported on 68 cases of meningococcic infection treated at Camp Edwards, Mass. Intravenous sulfonamides, especially sulfadiazine, were used. Not a single death resulted. The meningococcus was frequently recovered from clear spinal fluids.

Thomas[67] reviewed a series of 1518 cases treated in the Fourth Service Command. The early mortality rate of 8.8% in 317 cases was later reduced to 2.1%. Two-thirds of the cases and 80% of the fatal cases were noted in new, unseasoned troops.

The American experiences correspond quite well with those reported in British troops. For instance, Priest[63] reported the same "miraculous" results with the sulfonamide treatment of the 204 cases which occurred among the British Expeditionary Forces in France. Only 2 patients died, in neither case directly due to the effects of the meningococcus. In general, the cases were of 3 types: (1) fulminating onset with loss of consciousness and late appearance of meningeal signs; (2) acute onset with immediate signs of meningitis, the most common type; and (3) onset with chronic meningococcic bacteremia.

Prophylaxis. Thomas[67] has stated that the carrier rate is between 1 and 2% in non-epidemic periods and rises to 30% or more during epidemics. Gray and Gear[59] have expressed the belief that if the carrier rate exceeds 20% in a military camp, the mass use of sulfapyridine is indicated. Fairbrother[58] had previously demonstrated its effectiveness in the elimination of carriers. Sulfadiazine and sulfathiazole have proved themselves equally effective as prophylactic agents. Sulfanilamide apparently has not been as useful.[65] Kuhns *et al.*[61] administered sulfadiazine prophylactically to more than 15,000 soldiers. Following the administration of 3 gm. daily for 3 days or 2 gm. daily for 2 days, the incidence of cerebrospinal fever in the group fell abruptly. Only 2 cases occurred during an 8 week period of observation, while 40 cases occurred among 18,800 untreated controls. One of the 2 treated patients was admitted to the station hospital for measles and developed meningitis 9 days later; the other was transferred to an untreated organization after he had received the drug.

Use of Electroencephalography in the Armed Forces. Schwab[71] states that the usefulness of the electroencephalographic examination of navy personnel falls into 3 main categories: (1) in examination of applicants for naval service, the examination being confined to those men with histories of fainting attacks or head injury; (2) in examination of men actually in the service who have fainted or have had seizures; and (3) in determination of the extent of intracranial damage to members of the personnel who have suffered head injuries. Williams[73] studying an English

military group found 40% abnormalities among men with head injuries, 26% abnormalities in psychoneurotics and 5% abnormalities in a group of military aviators. Ten per cent of the records in a control group were classified as abnormal. Harty, Gibbs and Gibbs[68] have reported on the electroencephalographic examination of 275 candidates for military service. Thirty per cent of the records were abnormal, contrasted with 15% in a control group. However, when 127 men in the group with a history of severe head injury or neuropsychiatric disorder were eliminated, the percentage dropped to 13. The greatest percentage of abnormalities were found in psychopathic personalities (88%) and in head injuries with neuropsychiatric disorders (85%). In 6 candidates with a family history of epilepsy, 4 were abnormal, while in 4 with seizures in infancy 2 were abnormal. Of cases classified as "mixed neuropsychiatric disorders," 61% were abnormal. Among men with head injuries, 26% with a history of unconsciousness following the injury had abnormal records, while but 11% were abnormal in those in which consciousness was unimpaired. The authors concluded that electroencephalographic examination is advantageous in cases in which the results of other tests are equivocal or in which a general examination indicates that a man is borderline between acceptable and rejectable.

In chronic post-traumatic syndromes, Heppenstall and Hill[69] relate the abnormalities in electroencephalographic findings to the duration of post-traumatic amnesia, the time elapsed between the injury and the recording, and to abnormal family and personal histories. Sixty-three per cent of the records were abnormal in cases of predominantly "organic" syndromes and 37% in predominantly "functional" syndromes. Denny-Brown[12] states that improvement in the electroencephalogram in cases of concussion corresponds roughly with the clinical condition of the patient. The record may not be of value, however, if it is unrelated to clinical data.

McDaniel *et al.*[70] report on the use of the electroencephalogram at the U. S. Naval Hospital in Philadelphia. They state that it is proving itself at least as useful to the neuropsychiatrist as the electrocardiogram is to the cardiologist. Solomon *et al.*[72] also believe electroencephalography to be a valuable instrument in the selection of recruits, especially those with the diagnosis of epilepsy. Among 432 patients, they state that the electroencephalogram was of primary importance in arriving at a decision in 10%, of secondary importance in 53% and of incidental value in 37%.

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PHYSIOLOGY

PROCEEDINGS OF
THE PHYSIOLOGICAL SOCIETY OF PHILADELPHIA
SESSION OF NOVEMBER 16, 1943

Determination of Serum Total Base by Ion Adsorption. B. D. Polis and J. G. Reinhold (Division of Biochemistry, Philadelphia General Hospital). A simple, rapid method for total base determination was

described based on the adsorption of the cations by Amberlite Resin 1R100 and the titration of the acid effluent with standard base. CO_2, which is partially lost, is removed by aëration with CO_2 free air. The base bound as bicarbonate is calculated by a CO_2 determination. This added to the value determined by titration gives the total serum base. The method showed high precision and good accuracy compared with the electrodialysis method of Keyes, except in the extreme acidic range of 0–10 meq. HCO_3. In this range the results, though low, are consistent enough to apply a correction factor.

The Blood Picture of Athletes as Affected by Intercollegiate Sports. EDMOND J. FARRIS (The Wistar Institute of Anatomy and Biology).* Over 300 blood counts were made on college athletes before and after match games. In most of the 8 sports an emotional lymphocytosis was evidenced before the game, with concomitant reduction in the neutrophils.

In football, basketball and baseball the relative lymphocytosis disappeared during the course of the game. In wrestling and track the lymphocytosis increased during the course of the event. In tennis, golf and rowing, the relative lymphocytosis remained about the same throughout the event. Some indication of the physical condition of each player was furnished by the differential blood count picture after football, basketball and baseball games. Blood counts of players fatigued to exhaustion ranged from 80 to 91% neutrophils.

Intensity of activity in competition, together with duration, produced a leukocytosis in all events. A certain intensity in activity was essential to produce the marked leukocytosis, in spite of duration.

The total number of erythrocytes per c.mm. was increased in the short term athletic events, and usually decreased in events which lasten over 25 minutes.

Clues Regarding the Organization of the Simplest Living Systems. STUART MUDD (Department of Bacteriology, University of Pennsylvania). The microbiologic parasites may be arranged in a series of diminishing size and metabolic independence. Bacteria are able to synthesize certain growth accessories, lipids, carbohydrates, nucleic acids and proteins. It must be admitted that no pattern of organization of sufficient refinement and complexity has as yet been detected as a possible structural basis for these exacting chemical syntheses. Nevertheless, electron microscopy has shown a considerable degree of organization of the minute bacterial cell. A cell wall in the solid state is separable from an inner fluid or potentially fluid protoplasm. Extracellular capsules, flagella, spores and spore-like bodies, intracellular granules and areas of differing densities are all, when present, capable of demonstration under favorable conditions.

Pathogenic rickettsiæ and viruses have either lost or never achieved their metabolic independence. They are obligatory intracellular parasites. The studies of Plotz, Smadel, Anderson and Chambers with rickettsiæ and of Green, Anderson and Smadel with vaccinia (which is one of the largest viruses), nevertheless demonstrate simple cellular organization. Cell walls and differentiation within the protoplasm are clearly seen in these rickettsiæ and in the vaccinia elementary bodies. Structural differentia-

* This investigation was aided by a grant from the Samuel S. Fels Fund.

tion, indeed, has been recorded by Luria, Delbrück and Anderson in micrographs of even smaller parasites, namely in the heads of coli bacteriophage particles, which are only about 65 to 80 mμ in diameter.

Plant viruses would seem to be at the bottom of the microbiologic scale of parasitism. The viruses of tobacco and cucumber mosaic disease have been prepared by W. M. Stanley and others as crystalline macromolecules of nucleoprotein. Analysis by Roentgen ray diffraction by Bernal and Fankuchen has shown the molecules of tobacco mosaic virus to have a definite internal architecture due to regular periodic arrangement of the structural units of which the macromolecule is composed. Tobacco mosaic virus possesses the property of reproduction in suitable hosts and of "mutation," including change in chemical constitution; these are two attributes which have hitherto been considered distinctively characteristic of living systems. It may well be questioned, however, whether a crystalline molecule can be considered to be living.

An Apparatus for Study of the Physiology of Air-borne Infection. W. F. WELLS (Laboratories for Study of Air-borne Infection, University of Pennsylvania). In experiments on air-borne tuberculosis the number of tubercles in the lung corresponded with the estimated number of bacilli-bearing nuclei breathed by rabbits, and their general distribution indicated ready penetration to the remotest passages. The physical behavior of air-suspended particles, which determines their penetration to the lung, is governed by the relation between surface area and weight. Friction (proportional to surface area) holds particles in air currents, while mass deposits them on surfaces. Their state of suspension can therefore be defined by settling velocity (measured by the number of particles which settle on unit area in unit time divided by number per unit volume).

The solid content of a droplet determines the size of its particulate residue. Settling velocity of nuclei can be regulated by the solid content of droplets of constant size produced by a special atomizer. The atomizer nozzle inserted tangentially at the equator of a horizontal flask whirls larger droplets to the flask wall where they return to the pool. The air is rapidly saturated by the evaporation of a constant number of the smallest droplets, and their nuclei are swept out with the air stream frost the core of the flask air. The constancy of both size and number depends upon the saturation of a constant supply of air of constant humidity by droplets of constant size. Loss in flask weight determines the amount of atomized fluid, gauge readings the amount of compressed air used, and bacterial sampling the number of droplets. Settling velocity (or particle size) is estimated from simultaneous Petri plate and centrifuge samples.

The number of particles in the air breathed by the animals is maintained constant by chimney action—draft being created by a special gas burner supplied by the infected air. Any system connected to this chimney will be under negative pressure to the combustion chamber, reassuring safety of persons in the room. Air flow is estimated from readings of wet and dry bulb thermometers in the air line.

A hexagonal infection chamber is connected by a 2-inch hose line to the chimney and to the atomizer flask. Each of the 6 sides is fitted with a port, to which a plethysmograph can be attached, so that the head of the

animal projects into the chamber. Influent air rises around the edges of a false floor, passes the heads of the 6 animals facing each other, and discharges through a central outlet, effluent air being sampled by an air centrifuge exhausting the outlet line. A small ultraviolet light sterilizes the air at the termination of exposure and reduces superficial contamination of the animals and interior surfaces. A kymograph standing on top of the chamber records air displacemt. From the measurement of air breathed by the animals, and the bacterial content of the air, the number of inspired particles can be estimated, and by comparison with the number of colonies in the lung, the penetration of defensive barriers can be correlated with particle size.

BOOK REVIEWS AND NOTICES

BAROMETRIC PRESSURE. Researches in Experimental Physiology. By PAUL BERT. Translated from the French by MARY ALICE HITCHCOCK, M. A., Formerly Professor of Romance Languages at the University of Akron; and FRED A. HITCHCOCK, PH.D., Associate Professor of Physiology at The Ohio State University. Pp. 1055; 23 tables; 89 figs. Columbus, Ohio: College Book Company, 1943. Price, $12.00.

IT is the fate of most individual contributions to knowledge to be washed by the years into a heap of anonymity—many of them forever, and some to be long forgotten. The rescue of great classics depends not alone upon their greatness, but also upon that small, select group of alert minds who, not content to let the heap lie still, search out the Vesalii, the Harveys, and the Paul Berts for our illumination and inspiration. Paul Bert's narrow escape from these misty reaches of dim, forgotten time is due less to the global war which has brought the physiology of altered atmospheric pressure into prominence than to the timely perspicacity of the translators, to whom humanity must be grateful for the rebirth of a classic of orderly, scientific investigation during a time of utter chaos. In the preface of the original edition (1878), Bert does not exaggerate in saying, "The present book, if I am not mistaken" (and indeed he is not) "holds interest not only for physiologists, but also for physicians, engineers, and even travellers."

To touch now briefly upon the plan and structure of this *magnum opus* given us by the Hitchcocks, and to sample here and there its brightest passages will be, it is hoped, the *antipasto* which demands enjoyment of the whole feast.

The work is divided into 3 parts: history, experiments, and conclusions. Of the first part, some 500 pages, Dr. John F. Fulton says in his foreword to the translation, "We should be lastingly in (Paul Bert's) debt for this masterly presentation—a model, be it said, for any student wishing to write in the field of medical history." With this comment there can be no disagreement by the most casual reader of the book. One is led first upon a tour of the Lofty Regions of the Earth, from the Alps through the mountains of the Caucasus, Marco Polo's Roof of the World, the Himalayas, the peaks of the Americas and Africa, and the highest islands, with something of their history and measurements of their altitudes. With this orientation, the author gathers the observations of travellers on mountain journeys, quoting from the followers of Cortez in 1530, von Humboldt in 1802, and a host of others, from wandering Jesuit priests to Bert's own contemporaries. From these, and similar accounts of balloon aëronauts, he passes to a discussion of all the explanations and experiments devised to explain the symptoms suffered by those who ventured to high altitudes—symptoms which received an interesting variety of names, depending upon the age and the location. Among the earliest investigations were those of Boyle in 1670, which were followed by a dark age of mysticism and polemics lasting until the author's time, and capable of furnishing an admirable half-semester course on the questionable art of covering ignorance with polysyllabics. Of this period, it might be said again, "of the making of books there is no end, and much learning is a great weariness of the flesh." Even the illustrious von Humboldt, we are told, originated the picturesque theory that much of the disability of mountain sickness arose from the simple lowering of atmospheric pressure, which normally kept the head of the femur in its articulation, with the result that this joint was painfully weakened. This concept held much sway until Bert caused a dissection to be made, and with careful measurements, applied the simple laws of physics. By similar methods in his critical summary, the author pulverizes the unsound to extinction. The remainder of the historical section is concerned with pressures above 1 atmosphere; it contains a fascinating history of the art of

deep-sea diving in bells and suits, a topic which forms an excellent prelude to the first part of Hill's "Caisson Disease," published in 1912. In addition, one learns that the compressed air chamber was first used therapeutically in France in 1838—the *anlage* of the modern oxygen chamber. Again, the numerous theories and experiments devised to explain physiologic changes under compression are criticized and summarized, and the subject of decompression illness is given its first clear presentation.

It is frankly impossible to review or even comment justly on the second 500 pages, entitled Experiments, within a reasonable space. Of these there are some 600, presented with complete protocols (models for the modern laboratory), summaries and discussions. All the questions implied by the preceding history are undertaken for study, and the subjects used are legion: 10 species of birds, 6 of mammalians, including 11 breeds of dogs in addition to mongrels, 4 species of cold-blooded vertebrates, snails and earthworms, 14 of insects, 5 of plants, numerous microörganisms, and *in vitro* preparations of tissues and enzymes. Here one may find, clearly set forth, the basis of much of our present knowledge of the oxygen-carrying function of blood under various pressures. In summarizing his experiments on rapid decompression, Bert gives the key to decompression illness and the modern practice of deep-sea diving. "Sudden decompression, beginning with several atmospheres, brings on symptoms of varying severity depending upon the degree of compression, the animal species, the individuals, and the state of the experimental animal at the time. These symptoms must be attributed to the escape of nitrogen which had been stored up in excess in the organism, following Dalton's Law." As prophylaxis he prescribes "controlled decompression," and as the only cure "a recompression, either immediate or following the inhalation of oxygen in case heart gurgles are observed." But perhaps most important of all is his discovery of the phenomenon of *poisoning by oxygen*, when, in his own words, "The oxygen which has reached a certain tension constitutes a dangerous element, often even fatal, for animal life." It is interesting that this phenomenon was little explored for half a century after its discovery.

But to proceed further with this delightful task of sampling would be unfair. Suffice it to add, again in the words of Dr. Fulton, "The third and final part . . . . contains the résumé and conclusions, and is again a model of concise, orderly and logical scientific presentation."

Lest it be noted that in discussing a translation the Reviewer has devoted much space to enthusiasm over the original, it should be remembered that there would be no cause for review in the absence of a translation of an exceedingly rare volume. And the Reviewer hastens to add (having read both French and English editions), that they have skilfully reconciled the 2 most difficult tasks of translation: to render faithfully and still use the best idiom.

The service performed by Paul Bert speaks for itself; that performed by his translators cannot be too highly praised: as the "Introduction to Experimental Medicine" of Bert's predecessor, Claude Bernard, is a "must" upon the shelf of every aspirant to the rocky paths of medical research, now there is available a companion, equal "must," in the Hitchcocks' faithful rendition of "Barometric Pressure." B. R.

HYPERTENSION—A Manual for Patients With High Blood Pressure. By IRVINE H. PAGE, A.B., M.D., Director, Lilly Clinic, Indianapolis City Hospital. Pp. 80; 7 figs. Springfield, Ill.: Charles C Thomas, 1943. Price, $1.50.

This book is written expressly for the layman and proposes to give the hypertensive "patient an insight into his illness that he may be spared some of the dismay and alarm and avoid the quackery that will await him from every side." Perusal of the book will repay the clinician as well as benefit his patient. The writer explains the purpose of the various clinical procedures used in examining the hypertensive patient, and further explains in clear and

understandable terms, what hypertension is. Finally, he tells what can be done about it. The approach is simple, direct, yet surprisingly comprehensive. In a field filled with contradictions, the author has maintained a firm footing on the underlying pathology and physiology of the cardiovascular-renal complex. This book should be gratefully received by the worried and harassed hypertensive patient, as it guides him to a satisfactory way of living and to a happier outlook upon his daily existence. D. C.

SELF-TEACHING TESTS IN ARITHMETIC FOR NURSES. By RUTH W. JESSEE, Science Instructor at Bridgeport Hospital, Bridgeport, Conn. Pp. 111. St. Louis: C. V. Mosby Company, 1943. Price, $1.50.

THIS manual, intended for elementary nursing students, fits well into the accelerated nursing school program of today. Logically graduated and stimulating, it provides for the student's self-orientation and self-study. While a larger size of type and additional laboratory exercises might further enhance its practicability, this Reviewer finds it an exceptionally valuable tool or both student and instructor. H. F.

CYTOLOGY AND CELL PHYSIOLOGY. Edited by GEOFFREY BOURNE. Pp. 296; several figs. and plates. New York: Oxford University Press, 1942. Price, $6.00.

IT gives one a definite confidence in mankind and secures one's faith in Science to realize that this book was produced by British scientists during the course of the 1940–1941 air blitzkrieg on Britain. In the Editor's words, ". . . . there are probably no chapters of which part was not written within the sound of bursting bombs." In spite of the adverse conditions under which the work was done, an excellent book has resulted. The contributors are: J. R. Baker, H. Blaschko, G. Bourne, J. F. Danielli, E. S. Horning, W. Jacobsen, R. J. Ludford, J. H. Schulman, and J. D. White. Realizing that purely morphologic investigations of cells are giving way to interpretation of structure in terms of chemical composition and function, the writers have striven to produce a critical analysis of cytologic technique and the results of physicochemical investigation of cells. The cell surface and membranes, mitochondria, the Golgi apparatus, the nucleus and the enzyme systems are capably discussed and the final chapter, by Ludford, presents the pathologic aspects of cytology. At the end of each chapter is a bibliography and the book is fully illustrated with drawings and photomicrographs. The chief value of this volume is the compilation and correlation of work from many sources and the result is the most lucid and comprehensive presentation available of our modern concepts of the cell. D. C.

APPLIED ANATOMY OF THE HEAD AND NECK. For Students and Practitioners of Dentistry. By HARRY H. SHAPIRO, D.M.D., Assistant Professor of Anatomy, College of Physicians and Surgeons, Columbia University. Pp. 189; 173 illus. Philadelphia, London, Montreal: J. B. Lippincott Company, 1943. Price, $5.50.

THIS is the first book of its kind, to our knowledge, since Cryer's *Internal Anatomy of the Face*, published in 1916. It is "specifically designed to relate the anatomy of the head and neck to the various fields of dentistry" for students and practitioners of dentistry. The book contains 9 chapters beginning with the examination of the patient including surface anatomy of the face, oral cavity and neck. Next is considered the development and growth of oral and associated structures including some of the more common congenital and acquired malformations, such as harelip and cleft palate, of particular interest to the dentist. After a short chapter on the skull, which contains some

excellent roentgenograms, there is one of the best sections of the book on the muscles and their relation to facial expression, orthodontic applications, movements of the mandible and their rôle in mandibular fractures. A chapter on the temporomandibular articulation contains a good discussion of the anatomic variations of the components of the joint—ankylosis and subluxation. The neuroanatomic considerations deal with the nervous structures relating to the head and neck and correlate them with pain, anesthesia and motor nerve disturbances. The blood-vessels, lymphatics and salivary glands are well covered. The final chapter discusses the structures affected in maxillo-facial war injuries and the rôle of the dentist in their care correlated with that of the plastic and general surgeon.

The many photographs, illustrations and roentgenograms are excellent and the format of the text is well arranged. L. C.

ESSENTIALS OF PROCTOLOGY. By HARRY E. BACON, B.S., M.D., F.A.C.S., F.A.P.S., Professor and Head of the Department of Proctology, Temple University Medical School and Hospital; Head of Department of Proctology, St. Mary's Hospital; Consulting Proctologist, Rush Hospital, National Stomach Hospital, Douglass Hospital, Mercy Hospital, and Paul Kimball Hospital, Lakewood, N. J.; Fellow, International College of Surgeons. Introduction by CURTICE ROSSER, B.A., M.D., F.A.C.S., F.A.P.S., Professor of Proctology, Baylor University, Dallas, Texas. Pp. 345; 168 illus. Philadelphia, London, Montreal: J. B. Lippincott Company, 1943. Price, $3.50.

ALL the essentials of proctology are given in this admirably readable form. Information is sufficiently detailed to make it of value to the surgeon, yet concise enough to be of value to the student and general practitioner.

Embryologic, anatomic and physiologic considerations related to proctology are presented in a single chapter. An entire chapter is devoted to examination and diagnosis. Instruments and instrumentation are discussed.

Thorough discussion of diagnosis and treatment are given on the following subjects: fissure in ano, cryptitis, anorectal abscesses, fistula, tuberculosis, proctitis and sigmoiditis, pruritus ani, hemorrhoids, foreign bodies, injuries, tumors, lymphogranuloma venereum, inflammatory stricture of the rectum, venereal disease, prolapse and procidentia, congenital anomalies of sacrococcygeal region, papillitis, megacolon, and malformations. There are chapters on anesthesia and analgesia, and proctologic nursing.

Diagnostic and therapeutic concepts are presented in well-labeled illustrations. There are several excellent tables emphasizing points in the differential diagnosis of various anorectal and colonic diseases. L. T.

AN INTRODUCTION TO MEDICAL MYCOLOGY. By GEORGE M. LEWIS, M.D., Member of the American Dermatological Association, Inc.; Fellow of the American College of Physicians, of the American Medical Association and of the New York Academy of Medicine; Member of the New York Dermatological Society and of the Manhattan Dermatological Society; Associate Attending Physician (Dermatology), The New York Hospital; Assistant Professor of Clinical Medicine (Dermatology), Cornell University Medical School; Attending Dermatologist to St. Clare's Hospital; Visiting Dermatologist to Welfare Hospital, etc.; and MARY E. HOPPER, M.S., Research Fellow in Medicine, Cornell University Medical School. Second ed. Pp. 342; 77 plates (1 in color). Chicago, Ill.: The Year Book Publishers, Inc., 1943. Price, $6.50.

THE fact that this edition became necessary within the short period of 4 years after the first speaks for the popularity of this book. This is a new edition, not a revision. New materials have been introduced, commensurate

with fresh developments since the first edition, and it is clear that many shortcomings that are inevitable in any first edition have been corrected.

The authors have done well in keeping the contents confined to mycology in its laboratory aspects, and are to be complimented in keeping it up to date. It continues to be unique and adequate in its field and indispensable to the dermatologists. F. W.

KAISER WAKES THE DOCTORS. By PAUL DE KRUIF. Pp. 158. New York: Harcourt, Brace & Co., 1943. Price, $2.00.

HENRY J. KAISER evolved a plan of prepaid medical service for the workers in his industries on the West Coast. This plan, carried out by Dr. Sidney Garfield, for the sum of 7 cents a day voluntarily contributed by Kaiser's men, gives them complete medical attention. According to the author, who writes with almost exhausting enthusiasm, the plan is very successful and should "wake the doctors" (*i. e.*, those not already awakened) to the possibilities of medical care being equitably distributed among rich and poor alike in all sections of the country. This is a timely and controversial subject which makes interesting reading. J. L.

RECONSTRUCTIVE SURGERY OF THE EYELIDS. By WENDELL L. HUGHES, M.D., F.A.C.S., Hempstead, N. Y. Pp. 160; 36 plates. St. Louis: C. V. Mosby Company, 1943. Price, $4.00.

THE various procedures used in the plastic repair of eyelids are here illustrated by cases from the author's practice. The work was originally presented as a thesis for the American Ophthalmological Society. It gives a compressive review of the literature on the subject for reference and provides a good reference work for the surgeon who has many of these cases to do.

F. A.

NEW BOOKS

Laboratory and Clinical Studies from the Memorial Hospital for the Treatment of Cancer and Allied Diseases. New York City. Vol. XXIII, Parts 1 and 2. 1942. Price, 25¢ each.

Principles and Practice of Rehabilitation. By JOHN EISELE DAVIS, M.A., Sc.D., Veterans Administration Facility, Perry Point, Md. Pp. 211; 6 tables. New York: A. S. Barnes & Co., Inc., 1943. Price, $3.00.

Experimental Surgery. A Laboratory Guide for Undergraduate Students. By J. M. MCCAUGHAN, B.S., M.D., PH.D., Assistant Professor of Surgery, St. Louis University School of Medicine, St. Louis, Mo. Pp. 82; 32 figs. St. Louis: The C. V. Mosby Company, 1943. Price, $2.00.

The author has rendered an important contribution by preparing a detailed laboratory guide for the instruction of students in surgical principles and technical procedures. It is recognized that instruction of animal surgery sometimes appears to place undue emphasis on the importance of surgical technique without recognizing the indispensability of experience in diagnosis and surgical judgment, but the author's method of approach guards against this pitfall. The plan of instruction emphasized the fundamental considerations of inflammation and repair and opens the eyes of the students to the alterations in physiologic mechanisms which follow anatomic alterations. Each exercise concludes with a number of questions and extensive bibliography which tend to encourage thought as well as action. Since this manual is quite unique it should find wide usefulness in surgical teaching. J. L.

Experimental Biochemistry. By GEORGE D. ESSINGER, M.S., PH.D., Associate Professor of Chemistry, Chicago College of Dental Surgery, Dental School of Loyola University, Chicago, Ill. Pp. 108; 9 figs. St. Louis: The C. V. Mosby Company, 1943. Price, $1.50.

This abbreviated laboratory manual of Biochemistry is designed for a short course. Most of the usual qualitative tests connected with carbohydrates, fats and proteins are given. No quantitative experiments for blood are included and only a few for urine. The quantitative determination of calcium in enamel is described. The manual apparently is intended for use with students of dentistry and certainly would not be suitable for a course in biochemistry given to medical students.

J. J.

Prelude to Sanity. By S. GREINER. Pp. 164. Published and Distributed by Master Publications (Box 647), Ft. Lauderdale, Fla., 1943. Price, $3.00.

A highly provocative attack on current biologic views, that contains more words and theories than supportive evidence. It, however, should contain interest for those who like this type of presentation. E. K.

A Medical Bibliography. A Check-list of Texts Illustrating the History of the Medical Sciences. Originally compiled by the late FIELDING H. GARRISON, M.D., and now revised, with additions and annotations, by LESLIE T. MORTON, Librarian, St. Thomas' Hospital Medical School. Pp. 412. London: Grafton & Co., 1943. Price, £2.10s. net.

White Blood Cell Differential Tables. By THEODORE R. WAUGH, B.A., M.D., C.M., Pathologist-in-Chief, Royal Victoria Hospital; Associate Professor of Pathology, McGill University; Consulting Pathologist, Montreal Homeopathic Hospital, Montreal, Quebec. Pp. 126. New York, London: D. Appleton-Century Company, Inc., 1943. Price, $1.60.

This booklet of tables is successfully designed for the convenience of those having to report many "leukocytes and differentials" and laudably desiring to give the absolute rather than the relative (percentile) numbers of the different varieties. The 100 tables range from 10 to 10,000 leukocytes per c.mm., each giving the value for each percentage. Thus, on the page for 7000 white blood cells, 1% of a given variety obviously equals 70; and 37%, less obviously, 2590. Familiarity with the book is so easily acquired that where much of this work is done, it should quickly pay time and energy dividends. E. K.

The Human Eye in Anatomical Transparencies. Explanatory Text by PETER C. KRONFELD, M.D., Director of Education, the Illinois Eye and Ear Infirmary, Associate Professor of Ophthalmology, The University of Illinois; Assistant Professor of Ophthalmology, Northwestern University. Anatomical Transparencies by GLADYS MCHUGH, Medical Illustrator, University Clinics, The University of Chicago. Historical Appendix by STEPHEN L. POLYAK, M.D., Professor of Anatomy, The University of Chicago. Pp. 99; 53 black and white illus., 34 colored transparencies. Rochester, N. Y.: Bausch & Lomb Press, 1943. Price, $6.50 (limited distribution).

Pain. Proceedings of the Association for Research in Nervous and Mental Disease, December 18 and 19, 1942. New York. Vol. XXIII. Editorial Board, HAROLD G. WOLFF, M.D., Chairman; HERBERT S. GASSER, M.D., JOSEPH C. HINSEY, PH.D. Pp. 468; 116 illus.; 19 tables. Baltimore, Md.: The Williams & Wilkins Company, 1943. Price, $7.50.

Psychological Medicine. A Short Introduction to Psychiatry. With an Appendix *War-time Psychiatry.* By DESMOND CURRAN, M.B., F.R.C.P., D.P.M., Psychiatrist and Lecturer in Psychological Medicine, St. George's Hospital, and Honorary Psychiatrist to the Maida Vale Hospital for Nervous Diseases, London, etc.; and ERIC GUTTMANN, M.D., L.R.C.P. (ED.), Neuropsychiatric Specialist, Emergency Medical Service, etc. Pp. 188; 21 figs. Baltimore, Md.: The Williams & Wilkins Company, 1943. Price, $3.50.

The Hospital in Modern Society. Edited by ARTHUR C. BACHMEYER, M.D., Director, University of Chicago Clinics; Director, Hospital Administration Course, University of Chicago; and GERHARD HARTMAN, PH.D., Director, Newton Hospital, Newton Lower Falls, Mass. Pp. 768. New York: The Commonwealth Fund, 1943. Price, $5.00.

Medical Clinics of North America. Philadelphia Number (November, 1943). Symposium on Medical Emergencies on the Home Front. Pp. 280. Philadelphia, Pa.: W. B. Saunders Company, 1943. Price, $16.00 per year.

Elements of Medical Mycology. By JACOB HYAMS SWARTZ, M.D., Assistant Professor of Dermatology, Harvard Medical School and the Postgraduate School; Member American Dermatological Association, American Mycological Association; Dermatologist, Massachusetts General Hospital. Foreword by FRED D. WEIDMAN, M.D., Professor of Dermatological Research, University of Pennsylvania. Pp. 190; 80 illus. New York: Grune & Stratton, Inc., 1943. Price, $4.50.

Medical Genetics and Eugenics. Vol. 2. By R. RUGGLES GATES, PH.D., D.SC., LL.D., F.R.S., Marine Biological Laboratory, Woods Hole, Mass.; LAURENCE H. SNYDER, B.S., M.S., SC.D., Professor of Medical Genetics, The Ohio State University; and EARNEST A. HOOTON, PH.D., SC.D., Department of Anthropology, Harvard University. Pp. 60. Published by Woman's Medical College of Pennsylvania, Philadelphia, Pa., 1943. Price, $1.00.

This is the second volume of a series of lectures on Medical Genetics sponsored by an alumna of the Woman's Medical College. It includes 3 lectures given to the public in the spring of 1943 at the College of Physicians of Philadelphia by R. Ruggles Gates (Our Ancestors, Ourselves, Our Descendants); Laurence H. Snyder (Heredity and Modern Life); and Earnest A. Hooton (Human Heredity or Forbidden Fruit of the Tree of Knowledge). The names of the speakers guarantee the excellence of the pabulum provided. The third speaker, as usual, titillates his readers, like his audience, with thought-provoking as well as amusing "Hootonisms." Such a wit should command a high price on the lecture platform, aside from the widening applications and increasing recognition of the importance of human heredity. Curiosity raises the question as to whether the "a" in his first name was given at baptism, or added by its possessor after his literary style became manifest. E. K.

NEW EDITIONS

Directory of Biological Laboratories. With Buyers' Guide. Pp. 96. Published by Burns Compiling & Research Organization, 200 Railway Exchange Bldg., 80 E. Jackson Blvd., Chicago 4, Ill., 1943 (issued annually). Price, $3.00.

The 1943 edition of this annual publication identifies approximately 800 laboratories concerned with biologic, bacteriologic or biochemical investigations. Research laboratories are included, also commercial consulting laboratories, and those related to manufacturing processes, the latter embracing food and nutrition products, vitamin products, organic chemicals and pharmaceuticals, biologicals, glandular products, and so forth. Personnel and professional data are shown for the laboratories listed. The Buyers' Guide, occupying just one-half of the Directory, gives a useful classified list of sources of supply for apparatus, equipment, instruments, materials, and so on. E. K.

Pathological Histology. By ROBERTSON F. OGILVIE, M.D., F.R.C.P. (ED.), Lecturer in Pathology and Assistant in Forensic Medicine, University of Edinburgh; Senior Pathologist, Royal Infirmary, Edinburgh; Pathologist to the Leith and Deaconess Hospitals, Edinburgh; Examiner in Pathology and Forensic Medicine for the Triple Qualification. Second ed. Pp. 411; 235 photomicrographs in color. Baltimore: The Williams & Wilkins Company, 1943. Price, $9.00.

The Foot. By NORMAN C. LAKE, M.D., M.S., D.SC. (LOND.), F.R.C.S. (ENG.), Senior Surgeon and Lecturer on Surgery, Charing Cross Hospital; Surgeon Bolingbroke Hospital; Consulting Surgeon, Emergency Medical Service; Director of Studies, London Foot Hospital; etc. Third ed. Pp. 432; 136 figs. Baltimore: The Williams & Wilkins Company, 1943. Price, $5.00.

A Synopsis of Surgical Anatomy. By ALEXANDER LEE MCGREGOR, M.CH. ED.), F.R.C.S. (ENG.), Lecturer on Surgical Anatomy, University of Witwatersrand; Assistant Surgeon, Johannesburgh General Hospital. Fifth ed. Pp. 710; 696 illus. Baltimore: The Williams & Wilkins Company, 1943. Price, $6.50.

The Dysenteric Disorders. The Diagnosis and Treatment of Dysentery, Sprue, Colitis and Other Diarrheas in General Practice. By SIR PHILIP MANSON-BAHR, C.M.G., D.S.O., M.D., F.R.C.P., Senior Physician to the Hospital for Tropical Diseases, Royal Albert Dock and Tilbury Hospitals; Consulting Physician in Tropical Diseases to the Dreadnaught Seamen's Hospital, London; Director, Division of Clinical Tropical Medicine, London School of Hygiene and Tropical Medicine; Consulting Physician to the Colonial Office and Crown Agents for the Colonies; Consultant in Tropical Medicine to the Admiralty and to the Royal Air Force; etc. Second ed. Appendix by W. JOHN MUGGLETON, M.S.M., F.I.M.L.T., Technical Assistant. Pp. 629; 108 illus. (9 colored). Baltimore: The Williams & Wilkins Company, 1943. Price, $10.00.

Symptoms and Signs in Clinical Medicine. An Introduction to Medical Diagnosis. By E. NOBLE CHAMBERLAIN, M.D., M.Sc., F.R.C.P., Lecturer in Medicine, University of Liverpool; Physician to Out-patients, Royal Liverpool United Hospital, Royal Infirmary Branch; Visiting Physician, Smithdown Road Hospital, Liverpool. Third ed. Pp. 456; 346 illus. (19 colored). Baltimore: The Williams & Wilkins Company, 1943. Price, $8.00.

NOTICE AND INSTRUCTIONS TO CONTRIBUTORS

MANUSCRIPTS intended for publication in the AMERICAN JOURNAL OF THE MEDICAL SCIENCES, and correspondence, should be sent to the Editor, DR. EDWARD B. KRUMBHAAR, School of Medicine, University of Pennsylvania, Philadelphia 4, Pa. Articles are accepted for publication in the AMERICAN JOURNAL OF THE MEDICAL SCIENCES exclusively, except in the case of subsequent publication in Society proceedings.

MANUSCRIPTS should be typewritten on one side of the paper only, and should be double spaced with liberal margins. The author's chief position and, when possible, the Department from which the work is produced should be indicated in the subtitle. ILLUSTRATIONS accompanying articles should be numbered and have typed captions bearing corresponding numbers. For identification they should also have the author's name written on the margin or back. The recommendations of the American Medical Association Style Book should be followed. REFERENCES should be numbered and at the end of the articles, arranged alphabetically according to the name of the first author and should be complete, that is, author's name, journal, volume, page and year (in Arabic numbers).

RETURN POSTAGE should accompany all manuscripts but will be returned to the author if the manuscript is accepted.

NEW NOTICE TO CONTRIBUTORS AND SUBSCRIBERS

Activated by a directive from the War Production Board, we have changed the size of our type page for the "duration" to effect an economy in the amount of paper used. While there is a smaller number of pages, the amount of material has not been noticeably reduced.

We hope that any unpleasant effect produced by cutting down the margins will be accepted and approved by readers as a temporary war casualty. It is possible that more radical changes will have to be made later, but we are loath to change any more than absolutely necessary, a format that has existed practically unchanged since the Journal began in 1820.

For the balance of the war, 150 reprints will be supplied gratis. Covers will be omitted on all articles. In ordering additional reprints, we will supply in multiples of 150. This modification is for the same reason as the change of format, *i. e.*, conservation of paper.

NOTICE TO SUBSCRIBERS AND ADVERTISERS

We desire to secure several copies of the March and May 1943 numbers of this Journal, in order to comply with requests and need for replacements in long library "runs." The war situation has made it impossible to print extra numbers to supply this demand. We would be *very* grateful to anyone who would return to the Publishers any unmutilated copies of these numbers for which they have no further use, and we would be glad to repay postage.